PROGRESS IN CLINICAL
AND BIOLOGICAL RESEARCH
VOLUME 354B

EFFECTS OF THERAPY ON BIOLOGY AND KINETICS OF THE RESIDUAL TUMOR, PART B: CLINICAL ASPECTS

Editors: Joseph Ragaz
Linda Simpson-Herren
Marc E. Lippman
Bernard Fisher

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EFFECTS OF THERAPY ON BIOLOGY AND KINETICS OF THE RESIDUAL TUMOR, PART B: CLINICAL ASPECTS

Proceedings of an International Symposium on the Effects of Therapy on the Biology and Kinetics of the Surviving Tumor, Held in Vancouver, British Columbia, Canada, February 15–18, 1989

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Preface

Effects of Therapy on Biology and Kinetics of the Residual Tumor includes the pre-clinical and clinical manuscripts presented at the International Symposium on the Effects of Therapy on the Biology and Kinetics of the Surviving Tumor, in the spring of 1989 in Vancouver, British Columbia, Canada. An attempt was made to focus the symposium on (1) the curative potential of a given therapy, (2) the behaviour of the residual tumor surviving such treatment, and (3) the short-term and long-term consequences of a given treatment as it affects the host.

There are indications that the therapy spinoffs, most of them insufficiently known and virtually unresearched, vary greatly not only in a given tumor site but also among individuals with the same tumor stage and other similar disease characteristics. At one extreme, there are situations where therapy may not lead to any documented adverse effects and where the direct tumor cell kill leading to a measurable response or cure greatly overshadows the possible hidden side effects on the surviving tumor population. At the other extreme, however, there may be situations where growth advantage of the residual tumor population after therapeutic cytoreduction does occur and could be documented if monitored properly. In those instances, the overall long-term effects of therapy may be of little benefit or may even adversely affect the host, as compared to untreated situations.

Part A of the symposium proceedings, *Pre-Clinical Aspects,* reviews in detail kinetics; kinetics and treatment design; the role of growth factors; genetic aspects of tumor growth; immunity and metastases; and the role of the microenvironment, differentiation, and chemoprevention.

Part B, *Clinical Aspects,* reviews biological response modifiers; radiobiology; clinical aspects of therapy, including issues on dose intensity, timing, and intraperitoneal administration of chemotherapy; the role of autologous bone marrow transplantation; and the short-term and long-term side effects of therapy. This latter section includes such topics as induction of second malignancies, long-term effects of radiation of breast cancer, and the quality of life.

The editors were privileged to welcome a large number of the world's leading authorities to review the key questions on tumor biology and therapy. It is the hope of the organizers that the symposium and these volumes will not only raise questions and issues of fundamental importance on tumor growth and biology, but might also serve as a springboard for further research in this area.

The organizers would like to acknowledge the assistance of Donna Watts, Lou Cox, and Betty Fata, from Venue West, Vancouver, for their assistance in organizing the convention; the below mentioned sponsors and exhibitors for their grant support to the symposium; Adria Laboratories U.S. and Ciba-Geigy Canada for their assistance in the preparation of this book; David Noble, the CCABC librarian, for his assistance in the editing of manuscripts; Mary Naylor and Fiona Buss, for their secretarial assistance; and all the symposium speakers and delegates for their stimulating presentations and discussions.

Joseph Ragaz
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REVIEW OF THE EFFECTS OF THERAPY ON THE BIOLOGY AND KINETICS OF THE SURVIVING CANCER. PART II.

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INTRODUCTION

This two volume narrative provides a comprehensive review of the effects of therapy, and in particular focuses on the kinetics and biology of the tumor surviving a given treatment. Invited Symposium presentations, and subsequently chapters in these two volumes, cover essential aspects of therapy, reflecting fundamental issues of tumor kinetics and biology conceptually helpful in assisting treatment planning. While the first volume concentrates on issues such as kinetics, genetic aspects of tumor growth, differentiation and chemoprevention, the purpose of this second volume is to review biological response modifiers, radiobiology, questions on timing and dose intensity of therapy, the role of autologous marrow transplantation, and lastly, the short-term and long-term effects of cancer therapies. The last section includes presentations on induction of second malignancies, long-term effect of radiation in breast cancer and two papers discussing the issue of quality of life.

BIOLOGICAL RESPONSE MODIFIERS

No other cancer treatment modality has evolved more rapidly or has received more attention in the recent years, than biological response modifiers (BMR). This section reviews several key issues of the topic.

***TUMOR TREATMENT AND CYTOKINES (J.W. Schrader et al).** Biological response modifiers (cytokines), according to Schrader, are biological substances on which the survival of host target cells depends. Cytokines are not present under strictly physiological conditions, but are induced by stress factors such as trauma, infection, immunological stimuli, etc. Most cytokines interact with one another and virtually no single cytokine is acting in-vivo entirely on its own. While most cytokines are active in a paracrine (locoregional) fashion, some of them, such as erythropoetin, G-CSF and GM-CSF, interleukin, etc., act via serum on distant target cells in endocrine fashion. Also, many of them exhibit a phenomenon of autostimulation and autoregulation, i.e. acting on the cells which produced them. While the detailed mode of their action differs in each cytokine, most of them interact with receptors present on the target cells. Their role in tumor therapy is either direct (causing cell kill without a mediator), or indirect (via activating other target cells of immunity). Also, many malignant cells have a direct or indirect cytokine dependence, such as in instances of leukemia dependence on IL3, or on GM-CSF. Some malignant clones depend on multiple factors. Cytokines are also implicated in molecular events underlying a progression (transformation) towards malignancy. For instance, several groups of viruses, such as Abelson murine leukemia, initiate upon their insertion into the host cellular DNA, a release of growth factors, likely responsible, as mitogens, for the initial events in the progression towards the malignancy. Lastly, Schrader discusses the therapeutic use of cytokines, such as their blockade with monoclonal antibodies, or alternatively, inactivation of growth factor receptors on the target cells.

***TOWARDS IMPROVED CANCER THERAPY USING SUBCUTANEOUSLY ADMINISTERED HEMOPOETIC COLONY STIMULATING FACTORS (G. Morstyn, et al).** Morstyn discusses recent advances in cancer therapy using subcutaneously (s.c.) administered hemopoetic colony stimulating factors. Bone marrow suppression is the main limitation of chemotherapy, and hemopoetic growth factors, such as bacterially synthesized G-CSF and GM-CSF, have been shown to be effective in restoring the nadir of white cells suppression. In his introduction, Morstyn provides some data on pharmacokinetics of G-CSF and GM-CSF. The Australian group used s.c. injections of these cytokines, and ELISA with radioimmunoassay techniques recorded measurable serum levels of both factors after s.c injection of 3 micrograms/kg, same as the levels shown to stimulate bone marrow effectively in vitro. Both s.c. bolus injections as well as Cormed pump s.c. infusions were used to deliver adequate levels of the cytokines in the serum.

During constant infusion, previously higher serum levels subsequently fell in parallel with the elevation of neutrophil counts. Such a "consumption" of growth factors implicates their direct participation at the level of the target cells - a part of the homeostatic mechanism controlling the level of the target cells. Clinical studies: Morstyn reported on 31 patients given high dose Melphalan i.v. (25 mg/m/2) with s.c. G-CSF given either as a s.c. bolus or as infusion. With the s.c. infusion there was initially a temporary fall in WBC. Only later, after escalation of the dose and achievement of steady serum levels, a 6 fold elevation of neutrophil count was noted. In patients treated with i.v. Melphalan, G-CSF was able to increase neutrophils to 1 - 3 x levels even when started 8 days after Melphalan. Cellulitis after the s.c. administered growth factors was seen very infrequently (1/122 patients). After GM-CSF, the following additional side effects were seen: bone pains, skin rash, first dose allergy effects (hypotension, fever, etc.), thrombophlebitis, elevated liver function tests and hypoalbuminemia. Dose limiting toxicities were pericarditis, fluid retention and autoimmune thrombocytopenia. After G-CSF, the following were seen: bone pain, abnormal taste, increased liver function tests and elevated serum urate levels.

Morstyn subsequently reviews their phase II and III studies with G-CSF. In the first report, 13 patients receiving chemotherapy and G-CSF support are compared to 18 historical controls with chemotherapy alone. The following results were seen: 1.) Reduction of the mean time to neutrophil recovery (>500/cm/3) from 20 to 11 days ($p<0.001$); 2.) Reduction of the duration of antibiotics ($p<0.001$); 3.) Earlier discharge from the hospital. 4.) Reduction of morbidity and cost of autologous bone marrow transplants. Lastly, Morstyn indicates that the utilization of factors such as G-CSF and GM-CSF as a "cocktail" may permit reduction of their dose and side effects.

***THE BIOLOGICAL BASIS FOR INTERLEUKIN-2 ANTICANCER THERAPY (H. Silver et al).** Silver et al. discuss here the biological and therapeutic aspects of Interleukin 2. They describe the cellular effector of IL2, the T-cell natural killer cell, the stimulation of which results in the formation of activated LAK cell. Interleukin acts via receptors present on the target cell. Tumor infiltrating lymphocytes (TIL), representing also activated T-cells found in the vicinity of the tumor, may differ and be superior to LAK cells, and if administered, may track the tumor site more effectively. Interleukin 2 exhibits multiple interaction with other cytokines, and some of its action may be mediated by other lymphokines, such as gamma interferon. Also, induction of IL2 production by T-cells, and the subsequent macrophage stimulation, may act as a triggering cascade for release of additional cytokines. Subsequently, Silver et al. provide a brief review of animal and clinical studies.

Therapy of animal tumors with IL2 has shown a reduction of metastases, a documented dose response to IL2, and results of combination of IL2 with LAK cells superior to treatment with IL2 alone. Clinical studies using the original Rosenberg phase I and II technique to prepare LAK cells (harvest of leukocytes, exposure in vitro to IL2 x 5 days, and re-infusion) showed in 106 patients an objective response of 22% (8 CR, 15 PR). Best responses were seen in renal cell carcinoma and in melanoma (31 and 21% respectively). Toxicity of IL2 - LAK therapy include fever, chills, malaise, G.I. upset, anemia, thrombocytopenia, and potentially the most serious effect , the capillary leak syndrome. New approaches of IL2 therapy include effector enhancement with concomittant administration of TIL, combined with cytotoxics such as cyclophosphamide and DTIC, and a direct administration of IL2 interperitoneally, intraarterially, or into the tumor. Modification of the latter approach involves intrasplenic administration of IL2 administration of IL2, as conducted by Silver and Klasa.

***MECHANISM OF ACTION OF INTERFERON (H. Ozer).** In his summary, Ozer presents data on the mechanism of interferon action, and provides a review of pertinent information on interferons. Reviewing the subject, he indicates that as a group, interferons exhibit a definitive antiviral activity. They do augment the function of the T-cell natural killer, and exert a profound regulation of cytotoxic T-cell function. They also influence immunoglobulin production of B-cells, thereby indirectly increasing also T-cell helper activity. Vis-a-vis other epithelial or malignant cell lineage, they enhance expression of class I and II histocompatibility antigens, and exhibit a direct antiproliferative activity towards normal or malignant cells, both in-vitro and in-vivo. One of the most exciting, albeit not yet sufficiently explored activities of interferons include their effects on DNA and on induction of differentiation, such as seen in myeloid and lymphoid cells. They are known to inhibit in-vivo at least 3 oncogenes (myc, ras and src). Production of interferons is induced by several groups of biological agents, the best known being viruses and several classes of growth factors - cytokines.

Lastly, according to Ozer, it may be that all actions of interferons, such as antiviral, antiproliferative and anti-tumor activities, are all related to the inhibition of growth factors, interacting with interferons in a negative feedback loop.

RADIOBIOLOGY

The impact of radiation treatment on human malignancies, now spanning over more than five decades, cannot be underestimated. Radiation therapy alone, or combined with chemotherapy, is known to cure, or contribute to long-term control of most human cancer sites. The next three papers discuss in more detail the key issues of radiobiology, providing some insight into the pre-clinical and clinical research.

***THE GROWTH KINETICS OF CLONOGENIC TUMOR CELLS THAT SURVIVE RADIATION THERAPY (R. Kallman).** While some aspects of the question on tumor cell kinetics after radiation are controversial, Kallman presents evidence which indicates an increase in the growth of tumor surviving radiation. Questions as to the mechanism of such growth enhancement are raised and include a possibility of 1.) a shortened cell cycle; 2.) increased growth fraction; or 3.) reduced cellular loss. Methodology to examine these alternatives is provided. Measurement of intermitotic times of surviving clonogenes by the application of time-lapse cinemicrography provides simple method of obtaining cell kinetic parameters. Films are televised and can be viewed (frame by frame) on a video monitor. Films provide clear pictures of cell divisions, cell death, fusion, etc. These observations, stored in the computer, permit the distinction of long-term clonogenic survivors from those clonogenic cells limited to 2 - 4 cell divisions after radiation.

In the analysis of the films of irradiated murine malignant cells exposed to hyperfractionated radiation, Kallman indicates that results provide no suggestion that either fractionation or hyperfractionation might cause a shortening of cell cycle time of surviving clonogens. Rather, the mechanism for increased tumor growth after radiation is either increased growth fraction (conversion of GO into G1 phase), or a reduction of the cell loss. COMMENT: This is a unique technique enabling, for the first time, a direct ongoing visualization of cell division. The implementation of this technique could allow further expansion on many fundamental observations of tumor growth, kinetics and biology. It may also permit examination of effects of most kinds of therapies, spanning from radiation treatment to chemotherapy, and from immunotherapy to genetic manipulation and chemoprevention.

***MICROENVIRONMENTAL CHANGES IN IRRADIATED TUMORS (S. Rockwell, et al).** Additional aspects of radiation therapy, including microenvironmental changes in irradiated tumors, are discussed by Rockwell et al. They indicate, similarily to Bissel and Dvorak in the section on stroma and microenvironment from the first volume, that the microenvironmental heterogeneity constitutes important factors responsible for differing behaviors and responses to therapy. In regards to resistance, there are no genetic changes, according to Rockwell, known to occur in irradiated tumors responsible for resistance to radiation. Passaged irradiated cells were even more sensitive to additional radiation than unirradiated cells. Hence, with respect to resistance, there is no analogy between chemotherapy and radiotherapy. Intrinsic radioresistance - albeit a true phenomenon - is not due to genetic mutations developing during, or as a result of, radiotherapy. In contrast, tumor stroma and microenviromental factors may be of great importance, in particular the significance of oxygen on radiation therapy. The main reason for radioresistance is hypoxia, oxygen being the most effective radiosensitizer. Tumors with defective vascular beds have decreased blood flow and a reduced delivery of oxygen. With reduced oxygen, there is a reduction of aerobic glucose metabolism, an increase of lactate and a reduction of pH. These metabolic changes are principal causes for reduced cellular proliferation, decreased growth fraction, and hence an increased resistance to radiation.

In some systems, radiation, after initial depletion of aerobic cells, may induce changes in tumor blood flow, with a subsequently increased delivery of oxygen. Reoxygenation, initiated according to some experiments as early as within one hour, may thus enhance the growth fraction, and reverse the radioresistance. In this regard, trials with agents inducing reoxygenation (radiosensitizers) such as misonidazole, are underway in many centres at the present time and constitute the backbone of ongoing radiobiological research. In the last portion of their paper, Rockwell et al. expand on the metabolic changes determining radioresistance, in particular the low pH.

The principal cause for a reduced pH is increased formation of lactate under hypoxia. Varying ability of malignant cells to proliferate in acid environment may be a major factor determining the heterogeneity in tumor proliferation. Drugs that interfere with pH may, therefore, be useful in therapy. Increase of pH may improve radioresistance, and a reduction of pH may improve the cell kill effect of other treatment modalities such as hypothermia. The manipulation of the metabolic environment may thus significantly improve the therapeutic ratio of radiation treatment.

***THE NATURE OF TUMOR HYPOXIA: IMPLICATIONS FOR THERAPY (D.I. Chaplin et al).** In the next and last paper of the Radiobiology section, Chaplin discusses in more detail the nature of tumor hypoxia and therapeutic approaches based on the experience of oxygen metabolism. He also indicates that anoxic cells are up to 3 times more resistant than oxic clones, and provides some data on the pathophysiology of anoxia. Of particular importance is occlusion of tumor vasculature seen more prominently in parallel with tumor growth, and the increase of the distance of cells from the tumor vasculature.

Chaplin discusses treatment strategies based on the premise that hypoxia can be turned into benefit by administering, for instance, bioreductive agents reduced into toxic metabolites under anoxic conditions. Subsequently, the induction of hypoxia, by reducing tumor blood flow with vasoactive agents such as 5-hydroxytryptamine or hydralazine, activates the given treatment. Chaplin describes results of experiments measuring the magnitude of tumor hypoxia by a fluorescence imaging technique using Hoechst's dye injection. In this experiment, oxic cells stain intensely, and anoxic dimly. The technique, documents alterations of tumor blood perfusion, thereby providing evidence for fluctuation of tumor oxygen saturation, a function of vessel closure. In this regard, vasodilating agents such as nicotinamide, have been shown to increase the fractional cell kill of radiotherapy.

Lastly, Chaplin reports on results of his group's study in which hydralazine (induction of tumor hypoxia through systemic vasodilation and reduction of tumor perfusion), when combined with agent RSU 1069, activated by anoxia is made 3 - 4 times more toxic, compared to hydralazine alone. He concludes by offering further suggestions regarding therapeutic manipulations of hypoxia, in particular combining chemotherapeutic agents such as Chlorambucil and Melphalan with radiotherapy, and with agents inducing anoxia.

INTEGRATION OF DOSE INTENSITY,KINETICS AND TUMOR BIOLOGY IN PRETREATMENT PLANNING

No other single factor of treatment of malignant diseases has received more attention since the early days of cancer therapy than the concept of dose of intensity of a given treatment. Over the decades, Skipper, Schabel and their group have worked out the foundations of knowledge on dose intensity, obtained mostly from animal data, but subsequently shown to apply to most human malignancies. In the later years, Hryniuk and his group have revived the issue of dose intensity by developing a quantitative methodology to calculate the intensity of a proposed or a given treatment, and documenting from retrospective analysis that dose intensity of chemotherapy may be of importance in human solid tumors.

***INTEGRATING THE CONCEPT OF DOSE INTENSITY INTO A STRATEGY FOR SYSTEMIC THERAPY OF MALIGNANT DISEASE (W.M. Hryniuk).** In his paper, Hryniuk describes in more detail the basic concepts of the dose intensity. He initially reviews the original data and definition of the dose intensity (DI), and the calculation of DI as a function of a given chemotherapy in mg/m2/week. He emphasizes that the intensity of treatment is even more important than the scheduling. The therapeutic outcome, depending on the tumor properties and the sensitivity of nonmalignant tissues, is a function of the tumor cell kill versus tissue toxicity, both determining the possible delivered maximum of the desired DI. The main conditions for the development of curative regimens include selection of the most appropriate agents, delivery of optimal treatment intensity, and maximal total dose of the given drugs. Other parameters of importance, according to Hryniuk, include such factors as circadian rhythm, determining periods during the day when tumor cell turnover is higher or lower, allowing thereby, further improvements of the efficiency of a given therapy by appropriate timing. Also, availability of supportive therapies, such as cytokines, bone marrow transplantation and antibiotics play an important role. Essential for the development of curative high intensity regimens is the knowledge and definition of chronic and cumulative toxicities, such as cardiotoxicity in the case of Adriamycin, pulmonary fibrosis in the case of busulfan, etc., and the development of protective agents or analogue drugs providing improvement of the therapeutic ratio. Lastly, he indicates that eradication of residual tumor burden may in long-term, consist of the ability of chemotherapy to deal with kinetically slow or non-dividing cells surviving the dose intensive therapies. These clones exhibit, therefore, kinetical and not biochemical resistance, and may need introduction of maintenance therapy, given perhaps at longer intervals, reduced intensity but an increased total dose. Postinduction treatments, in addition to maintenance chemotherapy, depend on individual tumor sites, and should consist in addition to such suppressive treatments as hormones, also of new treatment modalities such as differentiating agents (retinoic acid, etc.), or biological agents augmenting immunological processes, such as interferones, other cytokines, monoclonal antibodies or autologous activated lymphocytes.

***MODELLING THE EFFECT OF CHEMOTHERAPY INTENSITY (C. Coppin et al).** Coppin et al. expand on the concept of dose intensity, discussing the basic issues of chemotherapy treatment, such as the total drug dose, rate of delivery, resistance, toxicity, growth delay, repopulation doubling time, etc. They provide data and graphs of the mathematical model of chemotherapeutic intensity. One of the main conclusions of authors' model is that the treatment intensity and the total dose cannot be separated. This feature, accordingly, represents an important distinction from the claim that intensity alone may be sufficient. Their discussion is accompanied by graphic and numeric illustrations of simulated treatment outcomes, taking into consideration, for instance, systems with or without resistant or partially resistant stem cells, and correlating dose intensity with resistance. They conclude by stating that the overall cell kill and growth delay, in addition to other factors, are a function of total dose and not intensity. However, the dose intensity determines the amount of drug required to achieve a given net cell kill, and the dose intensity may also influence the degree of drug resistance.

***COMMENTS ON KINETICS AND BIOLOGY OF THE RESIDUAL CANCER, AND ON THE RELEVANT THERAPEUTIC STRATEGIES BASED ON THESE PHENOMENA (J. Ragaz).** The recognition and the consequences of altered behaviour of the tumor surviving therapeutic intervention, the main theme of the Vancouver Symposium, plays an essential part of tumor biology. Fishers' introductory remarks of this session, as well as his paper in the first part of this Volume, acknowledge the importance of altered tumor kinetics after non-curative surgery. Ragaz, who initiated studies of preoperative chemotherapy for breast cancer as a result of these and similar observations, discusses in his paper some of these fundamental biological phenomena. The initial animal data documenting alteration of kinetics after cytoreductive surgery, are reviewed. There is now growing evidence of a systemic nature of tumor growth for control and of intra -, and intercellular communication within the total tumor cell burden. Such evidence has been supported recently by the documentation of the existence of systemic growth factors, possibly mediating these communications.

Reviewed here in more detail is also the pathophysiology of enhancement of the metastatic potential in kinetically more active cells, indicating the association of increased growth fraction with increased cell detachment, transvascular diapedesis, and possibly increased chance for mutational changes leading to resistance. The analogy of the behavior of tumor cells after therapeutic perturbation with the kinetic and functional changes after injury in non-malignant tissues, such as liver or kidney emphasized. These data indicate some similarity in the biological behaviour of non-malignant and malignant tissues.

The analogy of the behaviour of residual tumor after therapeutic perturbation, documented in animal experiments, may also apply to human malignancies where anecdotal rather than well researched data are available. The author indicates, that in the clinical setting, non-curative procedures, such as incisional biopsies and incomplete surgeries may deleteriously affect the system, and that preoperative chemotherapy may be of benefit, to be confirmed in randomized studies. Such effect is achieved not only by the reduction of the overall systemic and locoregional tumor burden at the time of surgery, but also by a possibility of a reduction of the dividing capacity, and a reduction of the metastatic potential, in clones suturated or partially injured by preoperative chemotherapy.

***INTRAPERITONEAL CHEMOTHERAPY FOR PERITONEAL CARCINOMATOSIS FROM COLONIC OR APPENDICEAL CYSTADENOCARCINOMA (P. Sugarbaker).** Sugarbaker describes the characteristics and therapeutic consequences of tumors spreading primarily transcoelomically, and not intravascularly, such as the cystadenocarcinoma of the bowel or ovary. He indicates that the intraperitoneal rather than vascular spread may connote tumors of different biology, requiring different pharmacological approaches. These include intraperitoneal rather than intravenous chemotherapy. He subsequently discusses the pathophysiology of locoregional intraperitoneal spread, and the pharmacokinetics of intraperitonally injected 5-FU and Mitomycin. He indicates that steady plasma levels after intraperitoneal administration of these drugs are achieved, and are equivalent or higher, compared to toxic levels needed to achieve a documented cell kill in-vitro. Also, a reduced systemic toxicity is seen compared to intravenous administration of these drugs. He subsequently discusses in more detail the treatment plan of intraperitoneal chemotherapy of a malignant pseudomyxoma peritonei. Initial responses, according to Sugarbaker's data, are in the neighbourhood of 90%, compared to 20% with systemic treatment. Improved access of chemotherapy to the locoregional tumor sites in the postoperative time period may be of great importance.

AUTOLOGOUS BONE MARROW TRANSPLANTATION

No other treatment modality pays greater tribute to the concept of dose intensity than the high dose chemotherapy and radiation treatments requiring assistance of bone marrow transplantation. Editors therefore decided to review the topic in more detail.

***AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR HEMATOLOGIC CANCER (G.L. Phillips).** Phillips discusses here the role of bone marrow transplantation in the hematological malignancies. He indicates, that in 1988 (one year) as many as 1500 autologous bone marrow transplantations (ABMT)were performed for hematological malignancies in North America, their success rate and improved experience evidently responsible for their increasing popularity. The syngeneic bone marrow programme antedated ABMT, serving as an idealized model, particularly for cases where graft versus host disease could be prevented. Allogeneic bone marrow transplantations, presently the most commonly performed transplantation technique, is preferred, providing a histocompatible donor can be found. Involvement by the tumor, and depletion of stem cells by prior chemotherapy, are the main tangible limitations of ABMT. Great emphasis of ongoing ABMT research is on conditioning (induction) regimens, and on the marrow purification (purging) techniques. Presently, impact of purging on the outcome of ABMT is not known, mainly because of the inability to distinguish between relapse from the host (ineffective conditioning - induction regimens) as opposed to the relapse from the autologous marrow (ineffective purging). The methodologies for purging include the in-vitro elimination of malignant cells while sparing the healthy stem cells, or the removal and a subsequent administration of normal hemopoetic cells - a process called a positive selection. Most commonly employed techniques for purging include in-vitro marrow exposure to chemotherapy (e.g. 4-hydroperoxycyclophosphamide), physical techniques (density gradients, lectins), immunological (monoclonal antibodies), or biological approaches.

Subsequently, Phillips reviews ABMT of individual hematological malignancies. Most frequent are non-Hodgkin's lymphomas, where many long-term CRs are seen in relapsed patients. While the question as to whether patients should be transplanted at first CR, as opposed to the time of the first relapse, has not been resolved, it is becoming evident that high risk features at diagnosis may identify appropriate individuals to be transplanted at first CR. As for Hodgkin's disease, prior bone marrow involvement is not a contraindication for the autologous transplant. The most popular chemotherapy regimens contain Carmustine, BCNU and VP-16.

AML is also one of the most frequently transplanted diseases. Durable remissions are seen even after the second or third relapse, providing CR is achieved, and adequate induction - conditioning regimens, with or without purging technique, are used. Also, there are indications that improvement of the effects of induction regimens of chemotherapy may be achieved after combinations with total body irradiation. In ALL, most purging techniques include immunological manipulations, such as with monoclonal antibodies, complement purging, or combinations of both. Results of ABMT for CML has been traditionally less successful, as karyotypic CRs were infrequent. As seen from Vancouver data (Eaves et al.), long-term cultures of CML bone marrow prior to engrafting were successful, and for the first time, a recovery of intact Philadelphia negative cells was achieved, greatly improving the chances for a successful outcome.

*DOSE INTENSIFICATION USING COMBINATION ALKYLATING AGENTS AND AUTOLOGOUS BONE MARROW SUPPORT IN THE TREATMENT OF PRIMARY AND METASTATIC BREAST CANCER: A REVIEW OF THE DUKE BONE MARROW TRANSPLANTATION PROGRAM EXPERIENCE (W.P. Peters).

In his paper, Peters provides a review of dose intensification therapy for breast cancer using a combination of alkylating agents and autologous bone marrow transplantation. He initially discusses several background issues essential in ABMT. Peters provides evidence for dose response of alkylating agents, with evidence for cross sensitivity of alkylating agents used commonly in ABMT regimens, such as BCNU, Cisplatinum, Thiotepa, Melphalan and Cyclophosphamide. Peters indicates that because of marrow rescue, the non-marrow organ toxicities, such as veno-occlusive disease, resistant hypertension, and cardiotoxicity, are the main limiting factors, to be improved by alteration of the alkylating agents. With the assistance of ABMT, dose intensity can be greatly increased, and using Hryniuk's calculation, the dose intensity of Duke's transplantation regimen was 12.8 of Coopers' regimen.

He subsequently discusses other methodologies to test and measure effects of dose intensity, such as the calculation of the area under the curve, or utilization of molecular dosimetry in which direct damage to DNA is measured. In the last portion of his paper, Peters reviews over 100 patients treated at Duke University Medical Centre with high dose Cyclophosphamide, Cis-platinum, Carmustine, and high dose autologous marrow transplantation. Results have shown a high complete remission rate, ranging from 27 - 68%, including patients with previously treated refractory disease, and a short-median remission duration of 5 months. However, 15% of patients with no prior chemotherapy had unmaintained remissions of more than 2 years' duration, and overall, a very rapid induction of remissions was seen with median time till CR of 18 days.

Toxicity was significant, with 22% of therapy related deaths. In the new generation of Duke's ABMT regimens, an induction treatment program is initiated with Adriamycin, 5-FU and Methotrexate (AFM), followed by consolidation with high dose Cyclophosphamide, Cis-platinum, BCNU, and with a subsequent surgery or radiation treatment to the pretreatment disease bulk sites. The preliminary analysis of this pilot study shows an overall CR of 68%, and at median follow up of 11 months, only 15% of patients with CR relapsing. In view of these results, a randomized study with or without induction chemotherapy with AFM is to be started, followed by high dose consolidation + ABMT. Also, a study of high dose chemotherapy + ABMT is being initiated for high risk stage II breast cancer (>10 + ve nodes).

***DOSE INTENSITY STUDIES IN BREAST CANCER - AUTOLOGOUS BONE MARROW TRANSPLANTATION (G.N. Hortobagyi et al).** In the last paper on ABMT, Hortobagyi et al., report on the M.D. Anderson experience with high dose intensity studies and the autologous bone marrow transplantation program. In the previous phase II studies, without ABMT, the M.D. Anderson data showed that patients receiving >100% of the intended dose had significantly higher responses, and a longer median survival than patients with <80% of the dose. They also offer comments on the previously randomized high versus conventional FAC dose study. In this trial, identical overall responses, durations of responses and survival rates were seen, the high dose regimen documenting only a faster achievement of CR.

Applying Hryniuk's method of calculating dose intensity, (D.I.) they showed that although the high dose regimen had in the first few cycles a higher dose intensity than the standard regimen, in the subsequent last cycles, the delivered D.I. of the intended "high dose" regimen was lower compared to the standard schedule. This was likely due to a more severe bone marrow stem cell depletion, due to high dose chemotherapy preventing, in long-term, to maintain the high dose intensity in the "high dose" regimen.

This analysis emphasizes the need to analyze not only the intended but also the delivered dose intensity, and also the need for bone marrow supportive treatment, providing truly high dose intensity regimens are to be delivered. These and other observations led to the implementation of autologous bone marrow transplantation program at the M.D. Anderson Hospital. In this, induction regimen of FAC is started for 6 months, and is followed by high dose consolidation regimen with ABMT, utilizing high dose Cyclophosphamide, Cis-platinum and Etoposide.

Fifty four ER negative or hormone refractory metastatic breast cancer patients are presently reported, out of whom 47 are evaluable. Of those, 58% had CR, with median time to progression of 55 weeks, and a 2 year DFS of 35%. Mortality data showed 4 out of 54 treatment related deaths. Alternate consolidation non-crossresistant regimen is presently explored, with high dose Mitoxantrone, Etoposide and Thiotepa, offering a possibility for combination with the first ABMT consolidation regimen. Hortobagyi et al. conclude advising caution with the interpretation of dose response data, and suggest that increase of the dose intensity for only a short time (such as seen in the single pulse therapy ABMT) will unlikely alter the natural history of disseminated breast cancer.

***USE OF GRANULOCYTE - MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) TO SUPPORT INTENSIVE CHEMOTHERAPY (H.G. Klingemann).** Further discussion of high dose intensity chemotherapy treatments with GM-CSF support continues with the paper of Klingemann. He reviews all documented and expected benefits of GM-CSF therapy used for such conditions as aplastic anemia, myelodysplastic syndrome, stimulation of megakaryocytes, enhancement of neutrophils after chemotherapy, acceleration of ABMT engraftment, and also conditions such as radiation accidents and AIDS. Stimulation of bone marrow stem cells is considered the principal mechanism of GM-CSF, but some evidence for enhancement of other cell lines, such as solid tumors, is also available. According to Klingemanns' interpretation of results of the Dana Farber Institute using chemotherapy with or without GM-CSF, the superior results in patients with GM-CSF may indicate an increase of tumors' S-phase fraction, a result of stimulation with GM-CSF, suggesting the kinetic synchronization as yet another possible benefit of this cytokine.

In the last part of his discussion, Klingemann discusses the evidence for GM-CSF enhancing the functions of immune effector cells. In this regard, the increased phagocytosis and chemotaxis, enhanced anti-tumor and anti-viral cytotoxicity, increased oxidative metabolism and antigenic processing, all attest to the activation of monocytic function by GM-CSF. Induction of cytotoxicity in monocytes is mediated by other cytokines, such as tumor necrosis factor and interferons. Priming of monocytes with gamma interferon in cultures enhanced the cytotoxicity of monocytes treated with GM-CSF, and these lymphokine activated monocytes were effective against leukemia. Combining other lymphokines with GM-CSF, such as IL3, etc., has been started, and results indicate a further potential for improvement of GM-CSF therapies.

CONSEQUENCES OF THERAPY

***INDUCTION OF SECOND NEOPLASMS BY THE TREATMENT OF MALIGNANT DISEASE: LESSONS FROM HODGKIN'S DISEASE (J. Connors).** In parallel with increased curability of cancer, the long-term treatment toxicities, such as induction of second neoplasms, will inevitably rise. Most curative regimens of human malignancies, Connors, include radiation and alkylating agents, known carcinogens in animal systems. In his time-mortality assessment of Hodgkin's disease, Connors offers a more detailed review of the subject. He indicates that in the first decade after diagnosis, virtually all excess mortality is due to Hodgkin's disease itself or the immediate treatment related toxicity (sepsis, other infections, etc.) After the 10th year, the major contributions to excess mortality are late complications of therapy. After the 14th year, they are due to second neoplasms, most of them solid tumors. On the other hand, the onset of induced leukemia is seen much earlier, in the third to sixth year after treatment, decreasing to the background levels after 12 years.

In regards to the impact of the type of treatment, Connors indicates that the cumulative dose of alkylators is of importance, and that the addition of radiation to chemotherapy, particularly to alkylating agents, nearly doubles the risk of AML. Radiation alone, on the other hand, makes only a small contribution, and a combination of radiation with non-alkylators results in no increase in the leukemia incidence. Details correlating the type of treatment with induction of solid tumors are less clear, but the association of malignancies such as thyroid carcinoma, sarcoma, lung, breast and ENT carcinomas with prior-site radiation, has been documented. Several, but not all, second malignancies are more frequent if prior treatment of Hodgkin's disease started at a younger age. With increased long-term cures of Hodgkin's disease, increased incidence of second malignancies is already seen, and according to a recent update, is as high as 17.2% overall, with 13.2% solid tumors at 15 year follow up. In the net balance, the improved overall survival, taking into consideration treatment induced mortality including that from second neoplasms, still favours implementation of therapy for Hodgkin's disease. However, the very existence of induced tumors calls for caution against overtreating, and for a need to reassess the incidence of second malignancies in long-term follow up.

*** OVERVIEW OF ADJUVANT RADIOTHERAPY FOR BREAST CANCER. (J. Cuzick).** This paper provides a review of long-term effects of radiation therapy for breast cancer. Over ten thousand patients with breast cancer, of which 6000 died, are analyzed and reported here by Cuzick et al. The analysis includes trials of radical or simple mastectomy with or without postoperative radiotherapy. At the first 10 years, no effect of adjuvant radiation on survival is seen. After 10 years, at least some form of radiation had a deleterious effect on survival - the overall death rate after 10 years in the 10 year survivors was 35% higher in patients randomized to radiotherapy. Cuzick subsequently discusses some details of this data base. Firstly, because of the limitation of the initial questionnaire to only basic questions (randomization, age, nodal status, type of surgery), other important questions, such as type of radiotherapy, cause of mortality (cardiovascular death, secondary leukemia, other secondary tumors), are not available. These questions, according to Cuzick, are being answered in the follow up study, using a more extensive questionnaire. Results of individual trials contributing towards the overview, seem to point towards an excess of deaths from heart disease, particularly in patients with radiation of the left chest wall. The follow up of this study will be available in the near future.

QUALITY OF LIFE

***MEASURING QUALITY OF LIFE (D. Osoba).** In his paper, Osoba reviews the main concepts and data on assessment of life quality of cancer patients. Until the advent of effective cancer therapies, the quality of life was affected mainly by the disease alone. Lately, with the emergence of intensive therapies, quality of life has been significantly influenced, in both positive and negative way, by the applied treatment. Accordingly, assessment of the overall quality of life will have to integrate disease symptoms with all other therapy impact. This should include the positive spinoffs of therapy such as responses, overall survival, and palliative effects of therapy, combined with all the treatment induced side effects. These assessments could thus provide an estimate of the quality adjusted life years survival - a single numerical expression analyzing the cost/benefit of a given treatment, which could be subsequently compared among patients within one study or several participating groups.

Osoba also emphasizes that before the development of the technique for quality of life determination, as a primary step, the quality of life has to be properly defined, and such definition will subsequently enable identification of methodologies to assess the life quality adequately. In the definition of the overall quality of life, there is a need to incorporate at least three dimensions, the physical, the psychological, and the social one. Osoba subsequently elaborates on why quality of life should be measured, and who should do it. He also discusses the methodologies, such as the interview technique or self-report methodology. The latter methodology is a more uniform and comparable technique, more suitable for comparative assessment of patients in larger groups. All assessment techniques should be constructed to ensure reliability and inter-institutional reproducibility. Lastly, a number of currently available self-assessment questionaires are briefly reviewed.

***CHEMOTHERAPY SCHEDULES: IMPACT ON TREATMENT OUTCOMES AND QUALITY OF LIFE (M. Tattersall).** In the last part of this section, Tattersall discusses the impact of chemotherapy schedules on treatment outcome and quality of life. He emphasizes that despite treatment induced toxicity, the impact of chemotherapy, through the cell kill and remission induction, may positively affect the overall quality of life. The overall therapeutic ratio will be a function of the tumor cell kill determining the responses and overall palliation, versus the toxicity and duration of chemotherapy. Tattersall subsequently reports results of two Ludwig Breast Cancer Trials Group. The first one was testing the value of salvage chemotherapy, the same as given in the adjuvant setting, started again at the time of relapse. The second one was analyzing the impact of the duration of chemotherapy in patients with metastatic breast cancer treated with induction chemotherapy until response, plus three more cycles, followed subsequently by continuous versus intermittent chemotherapy. The second study also had a detailed quality of life assessment.

Results of the first study showed that re-treatment with CMFP regimen of patients who relapsed the same adjuvant treatment resulted in many responses. Hence, the study confirmed, similarly to past trials such as Bonadonna's, Jones or Buzdar's, the ability of adjuvantly pretreated patients to respond to the same therapy. These observations imply that the tumor relapse, particularly in patients relapsing more than 6 months after the completion of adjuvant treatment, does not connote an absolute resistance to the same regimen. Such a state may imply a "relative resistance", likely due to kinetic phenomena (non-cycling vs cycling state), and not necessarily end-stage resistance. Analysis of the second study has shown that all disease parameters, including the responses and survival, favoured the continuous arm. Furthermore, in the intermittent arm, the anxiety increased significantly during the periods without chemotherapy, and all the quality of life scores, found to be independent predictors of survival, also favoured continuous treatment.

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TUMOR TREATMENT AND CYTOKINES

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INTRODUCTION

In the past decade, developments in recombinant DNA, biochemical and biological techniques have resulted in the molecular characterization of a large number of soluble polypeptides that regulate the growth and differentiation of various cell types and can be broadly termed "cytokines". Cytokines have been characterized using various bioassays and have become known by variety of names based upon the bioassay or upon initial ideas about their actions or biological significance. These include "growth factors" e.g. platelet-derived growth factor, T-cell growth factor, B-cell growth factor(s), mast-cell growth factor, names reflecting supposed functions e.g. transforming-growth factor-α, transforming-growth factor-β, or names reflecting the way in which they were originally assayed e.g. the colony-stimulating factors (CFS's) named according to their principle target lineages as granulocyte-CSF (G-CSF), granulocyte-macrophage CSF (GM-CSF) or macrophage CSF (M-CSF, also known as CSF-1) or interferon-γ. There have been attempts to systematize the nomenclature resulting, for example, in the interleukin series which now has 7 members.

These substances have often been regarded as growth factors but it is clear that in most if not all cases they also can influence the function or state of differentiation of mature non-dividing cells. For example a factor such as interleukin-3, not only stimulates pluri-potential hemopoietic stem cells and progenitor cells to divide and generate differentiated cells but also causes mature cells such as macrophages to enhance their phagacytosis of bacteria (Schrader, 1988). In the case of certain cells of hemopoietic or lymphoid origin there is evidence that in vitro, and at least in some anatomical situations in vivo, the very survival of cells may depend on the presence of particular cytokines. For example hemopoietic progenitor cells and the blast cells of acute myeloid leukemia die rapidly in vitro in the absence of factors such as IL-3, granulocyte-macrophage colony stimulating factor or granulocyte colony-stimulating factor.

CYTOKINE RELEASE AS A STRESS RESPONSE

With the exception of erythropoietin, cytokines can be regarded as stress or emergency factors that are not present in normal individuals but are released only upon disturbances induced by trauma, infection or immunological stimuli.

Some cytokines are produced by a restricted number of cells - e.g. interleukin-2, interferon-γ, and interleukin-5 seem to be only produced by activated T-lymphocytes. Interleukin-3 and 4 are not only produced by activated T-lymphocytes but also by mast cells - one of the targets of these two cytokines - an example of an autostimulatory loop. Other cytokines such as IL-1, IL-6, GM-CSF or G-CSF are produced by a wider range of tissues. Epithelial cells, endothelial cells, fibroblasts and macrophages - all cells involved in defence and repair responses - are common sources of cytokines. There is as yet no evidence that any of these cytokines play any role in the normal, steady state production or function of cells in a normal adult. To deal with one important example, there is no evidence that the constant self-renewal of the hemopoietic system is regulated by any of the known hemopoietic growth factors interleukin-3, GM-CSF, G-CSF or CSF-1. This is of particular relevance to the possible use of growth factor antagonists in the treatment of tumors or inflammatory conditions because it suggests that they would not depress normal hemopoiesis. It also opens the possibility that there exist novel molecules that regulate novel steady state hemopoiesis; these may be cell-bound rather than soluble but could in theory be modified for use as soluble agents for stimulating hemopoiesis.

SITES AND MODES OF ACTION

Cytokines are potent agents, in some case exerting biological effects at levels of picograms per ml (10^{-13} - 10^{-14}M). With a few exceptions cytokines act in the vicinity of the cell where they are released and can be considered as paracrine rather than endocrine hormones. Erythropoietin and perhaps interleukin-6 and G-CSF differ in this respect from other cytokines and can act via the serum as endocrine hormones. This difference is reflected in the fact that erythropoietin, interleukin-6 and G-CSF have relatively long serum half-lives - of greater than 4 hours - in contrast with other cytokines where the half-life in serum is about 30 minutes. It is not uncommon for cytokines to act not only upon neighboring cells but also upon the cell in which they are produced. This physiological auto-stimulation or auto-regulation should be distinguished from those pathological auto-stimulatory events involving the aberrant production of cytokines which can be involved in the oncogenic process and will be discussed later. It is worth noting that auto-regulatory effects of cytokines could in theory be mediated, not only by autocrine pathways in which the growth factor is secreted and then binds to a cell surface receptor on the producing cell, but also by intracellular mechanisms. The implication of the latter mechanism for potential for therapeutic interuption of pathological autostimulatory links will be discussed below.

STRUCTURE OF CYTOKINES

In general cytokines can be regarded as unrelated polypeptides ranging in apparent molecular weight from 7,000 to 15,000, in most instances being modified markedly by glycosylation. There is no compelling evidence that the large number of soluble polypeptides or cytokines that have now been described share structural similarities or membership of a common gene-family. There are a few exceptions to this lack of homology at the level of amino-acid sequence, e.g. the families of α-interferons or of the NAF or gro-related factors (Lindley, I. et. al., 1988, and Richmond, A. et. al., 1988) or of a few pairs of related molecules, namely tumor-necrosis factor α and β (lymphotoxin) or interleukin-1α and interleukin-1β. Also IL-6 and G-CSF share some broad structural similarities suggesting ancestry from a common gene.

A remarkable number of otherwise unrelated cytokines do share a common short motif of amino acids at their NH_2 termini (Schrader et al. 1987). This limited homology probably reflects convergent evolution driven by a selective pressure for the N-terminus of these molecules to interact with a common structure or family of structures e.g. an enzyme or a receptor involved for example in the tissue distribution of these molecules. There is no real indication of the function of this N-terminal segment however.

ROLE IN TUMOR THERAPY

Cytokines have a number of potential uses in the therapy of tumors. These can be broadly characterized under four headings, namely; (i) direct anti-tumor effects e.g. by interferons or tumor necrosis factor, (ii) indirect anti-tumor effects e.g. the actions of interferons, interleukins or colony-stimulating factors in stimulating anti-tumor activity of macrophages, granulocytes or lymphocytes, (iii) as adjuncts to other therapy e.g. the use of growth factors to stimulate hemopoiesis following high dose to chemotherapy or irradiation and (iv) the use of cytokine antagonists to prevent or neutralize the aberrant, oncogenic role of cytokines in certain tumors.

The direct and indirect anti-tumor effects of cytokines such as interleukin-2 and interferon-α have been discussed elsewhere in this volume and will not be dealt with further here. Likewise the very exciting data on the use of hemopoietic growth factor such as G-CSF or GM-CSF to stimulate the hemopoietic system after high dose chemotherapy have been discussed by Dr. Morstyn and Dr. Gabrilove.

GROWTH FACTORS IN ONCOGENESIS

Our interest in the possibility of a direct role of growth factors in oncogenesis began some years ago when we observed the spontaneous emergence of a variant of murine, IL-3-dependent cell-line (Schrader and Crapper, 1983). Unlike the parental line the variant was able to grow in the absence of an exogenous supply of IL-3. The parent cell line was not normal in so much as it had karyotypic abnormalities and was immortal; however it would not survive when transplanted into a syngeneic mouse, a result in keeping with the fact that a normal animal lacks detectable levels of IL-3 in the serum. In contrast when the autonomous variant that was producing its own supply of IL-3 was injected into a syngeneic mouse, it rapidly gave rise to a disseminated myelogenous leukemia. The serum of these leukemic mice contained readily detectable levels of IL-3.

This represented the first instance in which the aberrant production of an auto-stimulatory growth factor was directly implicated in the onset of an oncogenic phenotype. Shortly afterwards it was observed that the production of the v-sis oncogene was homologous to one form of platelet derived growth factor (PDGF) (Waterfield et al., 1983). Subsequently a large number of instances where a growth of a tumor can be directly correlated with the production of an auto-stimulatory growth factor have been documented. We have been interested in exploring the frequency with which myeloid leukemias show this growth factor-related mechanism and in possible therapies based on interupting auto-stimulatory growth.

MULTIPLE GROWTH FACTOR ABERRATIONS IN A SINGLE LEUKEMIA

In one very instructive example of the effectiveness of growth factor related mechanisms of oncogenesis, we observed three independent, growth-factor related mechanisms operating in the progression of a single disease. These experiments involved a murine monocytic leukemia, WEHI-274, which had arisen in a mouse infected with the Abelson and Moloney murine leukemia viruses. We took cells from a leukemic mouse and plated them out in agar, either with or without a source of interleukin-3. We observed that at cell densities less than 10^5 per ml leukemic colony growth only occured if interleukin-3 was added to the cultures. In that the leukemic cells depended on an exogenous source of a hemopoietic growth factor for growth in vitro, this murine leukemia closely resemble myeloid leukemia in humans. At higher cell densities a small number of small colonies of leukemic cells did grow in the absence of interleukin-3 (Schrader et al, 1987, and Leslie and Schrader, 1989).

We isolated a large number of clones of this leukemia some from cultures that had lacked exogenous growth factors and others that had been cultured in the presence of interleukin-3. All of these clones were leukemogenic when injected into syngeneic mice. However they could be divided into at least three groups on the basis of their responsiveness to and production of cytokines.

The first WEHI 274 clone showed a classical autocrine pattern of growth in tissue culture; thus only at high cell densities was there any cell growth in the absence of added exogenous growth factors. In the presence of hemopoietic growth factors such as IL-3, GM-CSF and CSF-1, however, the leukemic cells grew rapidly. The density-dependent pattern of growth in the absence of exogenous growth factors suggested that the cells themselves were releasing a factor which at high cell-densities effectively conditioned their own environment. This was indeed the case as medium conditioned by the high-density cultures contained a factor with all of the biochemical and antigenic properties of interleukin-3 (Leslie and Schrader, 1989). Moreover these cells could be shown by northern-blot analysis to contain a number of abnormally large RNA transcripts that hybridized with an IL-3 cDNA probe. Southern blot analysis of this clone revealed a pathological rearrangement of one allele of the IL-3 gene. Analysis of this rearrangement has indicated that a intracisternal A type particle had been inserted about 200 bp upstream of the normal IL-3 promotor (Leslie and Schrader, unpublished observations). The large aberrant RNA transcripts reflected initiation of transcription in the LTR of this intracisternal A type particle and read through into the IL-3 gene.

A second group of WEHI-274 clones lacked pathological rearrangement of the IL-3 gene and did not produce the aberrant IL-3 transcripts or IL-3. However these class II cells were able to grow in the absence of an outside source of growth factor; moreover medium conditioned by these cultures contained a hemopoietic growth factor which was not IL-3 but had the antigenic and biological properties of a second T-cell lymphokine, GM-CSF. Northern blot analysis in this case revealed aberrant, abnormally large transcripts that hybridized with the GM-CSF probe. Southern blot analysis revealed a rearrangement of one allele of a GM-CSF gene. These experiments indicated that progression of this leukemia had taken at least two distinct routes, one of which involved aberrant activation of an IL-3 gene and the other aberrant activation of the second auto-stimulatory cytokine, GM-CSF.

A third group of clones derived from the mouse with the WEHI-274 leukemia behaved even more interestingly. Unlike clones of the first two classes, clones of this third class neither grew nor survived in vitro at any cell density. In keeping with this observation the medium conditioned by these cells stimulated neither their own growth nor the growth of other hemopoietic cells. The fact that these class III clones did not produce any autostimulatory growth factors and failed to survive in vitro without a source of growth factors, but were none-the-less fully leukemogenic when injected in vivo, was paradoxical.

How could these cells which were unable to survive in vitro give rise to a leukemia in vivo? Examination of serum or ascites fluid from mice bearing these leukemias, resolved this paradox, indicating that the leukemic mice were producing a factor which stimulated the growth of the leukemic cells.

PARACRINE ONCOGENESIS

The observations on the class III clones of WEHI-274 suggested the existence of a paracrine mechanism of oncogenesis. The implication of these results was that the leukemic cells secreted a factor which, while not directly stimulating its own growth, caused neighboring cells to release a growth factor.

This hypothesis was supported by in vitro experiments which showed that these Class III leukemic clones released a factor which when added to cultures of fibroblasts stimulated the release of a hemopoietic growth factor. The nature of these two factors is under investigation; the first factor released by the leukemic cell may well be interleukin-1β and the factor released by the fibroblast which stimulates the growth of leukemic cells, GM-CSF.

MOLECULAR EVENTS UNDERLYING PROGRESSION OF A LEUKEMIA

There was direct evidence that all three classes of WEHI-274 clones had a common ancestor. Thus all the WEHI-274 clones shared a common pathological rearrangement of one allele of the c-myb gene that had been caused by the insertion of a defective Moloney provirus (Gonda et. al., 1988).

The history of this leukemia may be constructed as follows: infection with a replicating antigen, namely the Moloney helper virus of the Abelson murine-leukemia virus, resulted in chronic stimulation of the immune system, the release of hemopoietic growth factors and extensive stimulation of hemopoietic cells. One hemopoietic progenitor suffered a genetic accident in which proviral DNA was inserted into the c-myb gene. It seems clear that the activation of this c-myb gene was not sufficient to render this cell autonomous. Certainly cells of the third class of WEHI-274 clones that shared this aberration in the c-myb gene were unable to survive in vitro in the absence of an outside source of hemopoietic growth factor. It seems likely that the activation of the c-myb gene played a role in the immortalization process. Constitutive production of the c-myb product is thought to block differentiation of hemopoietic cells.

It seems quite clear that progression to leukemia involved three independent aberrations of cytokine production. In two cases this involved the independent activation of distinct, directly autostimulatory growth factors, namely IL-3 and GM-CSF. In the third case the product of the gene that was constitutively activated was not directly auto-stimulatory but resulted in the release of a leukemia stimulatory factor from neighboring cells.

THERAPEUTIC USE OF ANTI-GROWTH FACTOR STRATEGIES

These observations of three distinct examples in the evolution of one disease suggests that aberrations of cytokine production may be a relatively frequent mechanism for oncogenic progession. They raise the obvious question of whether interuption of auto-stimulatory or paracrine stimulation of tumor cells will be a useful way of halting tumor growth. We have approached this question using as a model system, the murine myeloid leukemia caused by aberrant activation of an IL-3 gene (Schrader and Crapper, 1983).

Our initial experiments have used preparation of antibodies capable of neutralizing IL-3 at very high dilutions. We have made the somewhat surprising observation that even with high concentrations of anti-IL-3 antibodies, and even in situations where the leukemic clone is secreting only small amounts of IL-3, it is extremely difficult to completely inhibit growth and survival of the leukemic cell which on other grounds would seem to be dependent on autostimulation by IL-3. To inhibit growth 90% it is necessary to use at least one hundred times more antibody than would be needed to block the growth of the parental non-leukemogenic line in the presence of exogenous IL-3. These experiments suggest that in this instance at least the bulk of the growth factor that is critical for growth of the leukemic cell is accessible to the antibodies and is presumably therefore extra-cellular. However the very high concentration of anti-IL-3 antibodies required suggests that the action of a growth factor that is secreted by a cell is more difficult to block than that of a growth factor from an exogenous source. One explanation is that in the leukemic cell the IL-3 and its receptor interact intracellularly during the synthetic process, but do not generate in an effective signal. The latter occurs only after the receptor-hormone complex has reached the cell-surface and has associated with other proteins necessary for signal transduction.

It might be predicted that much higher concentrations of anti-IL-3 antibodies would be required to disrupt a pre-formed IL-3-receptor complex. These results thus leave open the possibility that antibodies directed against particular parts of the hormone or the receptor (or antagonists with similar properties) may interfere much more effectively in autostimulatory or paracrine mechanisms of tumorgenesis than polyclonal antisera. Thus it is possible that a monoclonal antibody with particular properties e. g. an antibody that bound to receptor-bound IL-3 and upon binding induced a conformational change in IL-3 that disrupted the receptor-IL-3 complex, could be effective at lower concentrations.

ANTI-RECEPTOR ANTIBODIES

In other experimental models we have infected immortalized lines of IL-2-dependent myeloid progenitor cells with a retrovirus that directed the production of IL-2. All of the IL-2-producing clones isolated proved to have become leukemogenic. In this instance of an auto-stimulatory tumor we have available monoclonal antibodies against not only the aberrant cytokine (IL-2) but also its receptor. We observed that high concentrations of anti-receptor and anti-cytokine antibody reduced proliferation in vitro by more than 90%. Once again these experiments suggest that a growth factor that is produced by a cell has a particular advantage in stimulating that cell.

SUMMARY

In summary, molecular biology and advances in biochemical and biological techniques have led to the rapid characterization of a large number of cytokines. These are released in response to injury or invasion of the body and regulate the growth and function of a broad variety of cell types. Many cytokines are now in clinical trials and show promise in modulating the defence and repair responses of the body. The direct application of these cytokines to the killing of tumor cells remains problematic. However, it does seem likely that the use of these substances in a para-physiological mode i.e. in activating defence mechanisms and in particular stimulating hemopoetins may be a very important adjunct to more conventional means of tumor therapy such as chemotherapy or radiotherapy. The hemopoietic growth factors show particular promise here, although other substances such as IL-1 or IL-6 may find similar applications. In the future more information on the direct role of cytokines in the development of particular tumors may lead to the development and use of cytokine antagonists with effects directed at tumor cells or their environment.

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TOWARDS IMPROVED CANCER THERAPY USING SUBCUTANEOUSLY ADMINISTERED HEMOPOIETIC COLONY STIMULATING FACTORS

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INTRODUCTION

There is evidence that the dose intensity of chemotherapy can determine the clinical outcome. Since neutropenia and thrombocytopenia limit the maximum dose of some cytotoxic drugs that can be administered it seems likely that if we could ameliorate marrow damage by these drugs we could achieve a greater dose intensity and better anti-cancer effects (DeVita, 1985).

Even standard dose intensity, chemotherapy is associated with fever and infection in 10-20% of cycles. At present, if patients develop fever and neutropenia, admission to hospital, intravenous antibiotics, repeated cultures and antibiotic changes are often required. If the neutropenia and fever persist for more than 7 days there is a significant risk of fungal infection. If we could prevent or reduce the period of neutropenia it seems likely that we could reduce the morbidity, mortality and cost of chemotherapy.

It is clear that hemopoiesis is under the control of specific regulators. Many of these have been identified, genetically cloned and are now available for clinical trials (Morstyn and Burgess, 1988; Morstyn et al., 1988(a)) We have undertaken studies with two of these regulators (Morstyn et al., 1989(a,b)) : Bacterially synthesized G-CSF (produced by AMGEN, Thousand Oaks) and bacterially synthesized GM-CSF (Schering Plough, New Jersey). This manuscript will review our phase I, II and III experience with each of these factors administered subcutaneously.

PHARMACOKINETICS OF G-CSF AND GM-CSF

We have developed an ELISA assay for GM-CSF (Cebon et al., 1988) and have used a radio-immunoassay for G-CSF (supplied by AMGEN, unpublished). The major findings using these assays were that subcutaneous administration of G-CSF or GM-CSF produced rapid and sustained serum levels. Figure 1 compares the serum levels reached following a subcutaneous injection of G-CSF or GM-CSF. Patients received a subcutaneous injection of G-CSF or GM-CSF of 3μg/Kg and the figure shows the levels of G-CSF or GM-CSF reached in the serum in ng/ml. Levels of GM-CSF > 1ng/ml produce near maximal stimulation of marrow _in vitro_ and these levels were reached following subcutaneous administration of 3μg/Kg of GM-CSF. We also measured serum G-CSF during a study of continuous subcutaneous infusions of G-CSF using a Cormed ambulatory infusion pump. An interesting finding was that during continuous constant infusions of G-CSF, serum levels fell when the neutrophil count became elevated rather than remaining constant as would be expected during a continuous infusion. This observation suggested that elevated neutrophil levels were associated with enhanced elimination of G-CSF. This may be part of the homeostatic mechanisms controlling neutrophil levels.

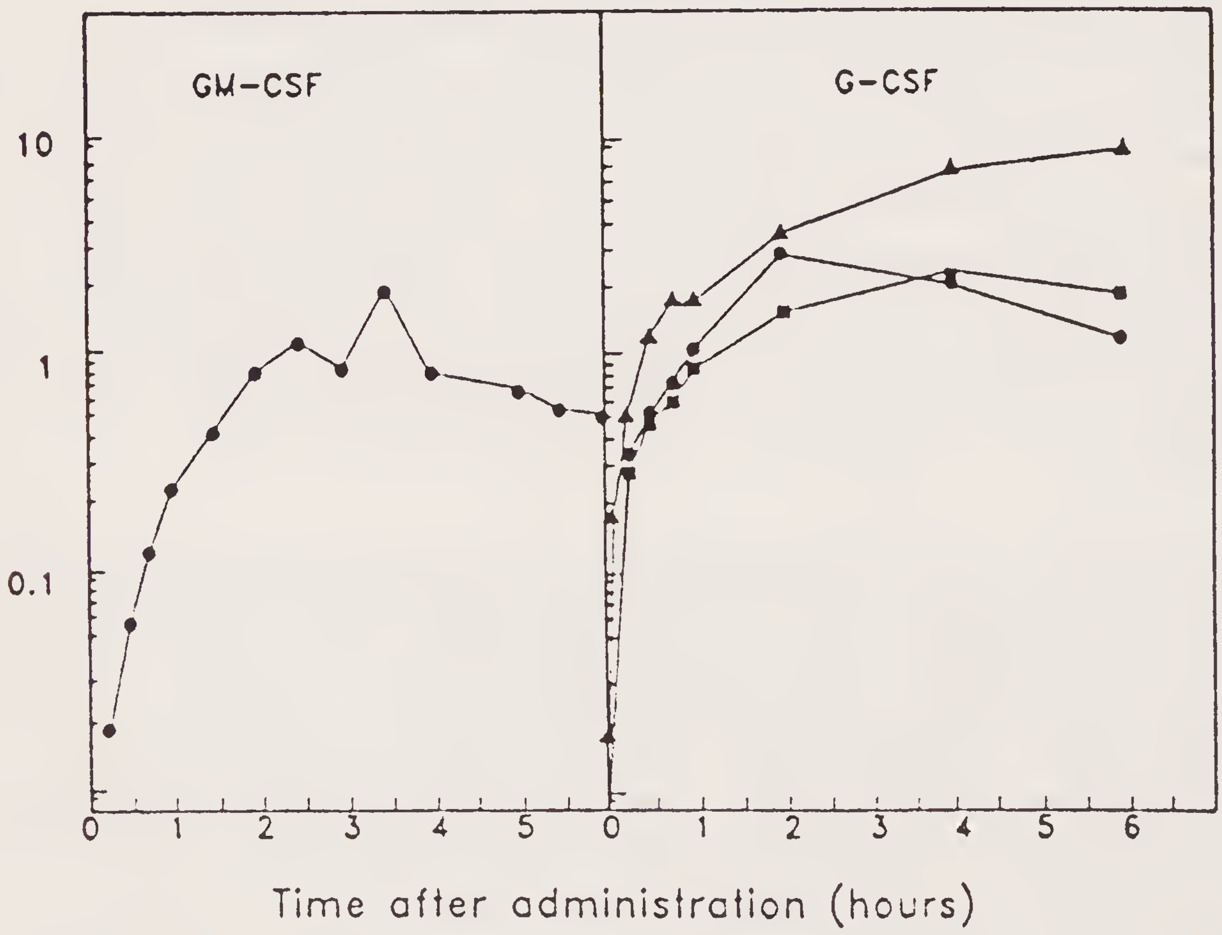

Fig. 1

ABROGATION OF NEUTROPENIA FOLLOWING STANDARD DOSE CHEMOTHERAPY BY G-CSF

We have studied 31 patients who have received intravenous melphalan 25mg/m^2 IV and subcutaneous G-CSF to abrogate neutropenia (Morstyn et al., 1988(c)). The first part of the study was a dose escalation study of G-CSF as a bolus at 0.3, 1 or 3 µg/kg/day subcutaneously. We also examined continuous subcutaneous infusions of 3 and 10µg/kg/day. As for IV administration of G-CSF the first effect of subcutaneously administered G-CSF was a transient fall in neutrophils in the first 2 hours. This was followed by a rapid sustained elevation in neutrophil levels and G-CSF administered at a dose of 10µg/Kg/day as a continuous subcutaneous infusion produced a mean 6 fold elevation in neutrophil levels. In patients who had not received extensive prior chemotherapy or radiotherapy doses of 3 or 10µg/kg/day subcutaneously were sufficient to abrogate the neutropenia caused by this dose of melphalan even when G-CSF was commenced 8 days after melphalan. G-CSF administered subcutaneously as a bolus or a continuous infusion was well tolerated. Only 1/22 patients receiving a continuous infusion developed cellulitis at an infusion site. The table shows a summary of the adverse observations we have made in our clinical studies of G-CSF. We chose the continuous subcutaneous infusion route of administration for subsequent phase II and III studies.

PHASE II AND III STUDIES WITH G-CSF

We are undertaking studies with subcutaneously administered G-CSF to determine if it can be used to ameliorate the neutropenia produced by high dose chemotherapy and autologous marrow rescue and to reduce the duration of fever and neutropenia in the setting of febrile neutropenia.

Thirteen patients treated with G-CSF and undergoing autologous marrow transplantation (Sheridan et al., 1989) were compared to a historical series of 18 patients. The patients had chemosensitive malignancies such as lymphoma, germ cell tumor and acute lymphatic leukemia. In the historical series there were also patients with lymphoma and germ cell tumor, acute lymphatic leukemia and with acute non-lymphatic leukemia. All patients received oral busulphan 4mg/kg/day for 4 days and cyclophosphamide 60mg/kg/day for 2 days. The major finding in the first 13 evaluable patients was that the mean time to neutrophil recovery (500 neutrophils per cmm) was 11 days whereas in the historical series it was 20 days ($p < 0.001$). Patients receiving G-CSF were on antibiotics for a shorter period ($p < 0.001$) and were discharged from hospital earlier. All patients however developed fever by day 10. There was a small but statistically insignificant reduction in the number of days to platelet transfusion independence. G-CSF produced a major decrease in the morbidity and cost of the autologous marrow transplants. To further improve outcome we need strategies to abrogate the initial period of neutropenia. We have recently demonstrated that G-CSF administration causes a marked elevation in the number of progenitor cells (Dührsen et al., 1988) in the peripheral blood. It is possible that these progenitor cells could be collected and infused to further reduce this period of neutropenia since progenitor cells in the recovery phase after chemotherapy are suitable for this purpose (Juttner et al, 1985).

ADJUVANT G-CSF IN PATIENTS WITH FEBRILE NEUTROPENIA

Apart from using G-CSF or GM-CSF to prevent neutropenia and episodes of infection it is possible that the CSF's could be used as part of the treatment in patients with chemotherapy induced neutropenia and infection. We are undertaking a randomized study of adjuvant G-CSF administered subcutaneously to patients who are on various chemotherapy regimes and who develop neutropenia and fever. The study is in 2 parts. In the first part all patients receive standard antibiotic therapy and half of the patients are randomized to receive G-CSF. The second part is an open label study in which all patients with a second episode of fever and neutropenia receive G-CSF. It is too early to analyse the randomized portion of the study. The first 6 patients entered onto the open label part of the study all achieved a rapid elevation of neutrophils to 500/cmm at a mean of 3.3 days, 1000/cmm at a mean of 4.0 days and 10,000/cmm at a mean of 6.3 days. It therefore seems as though it is feasible to rescue patients from neutropenia by the use of G-CSF. Further patient accrual and follow-up is required to determine the actual benefit as judged by the number of febrile days, time on antibiotics or time to discharge from hospital.

ABROGATION OF THE NEUTROPENIA CAUSED BY CARBOPLATIN (CBDCA) AND VP16 USING SUBCUTANEOUSLY ADMINISTERED GM-CSF

In extensive phase I clinical studies of bacterially synthesized GM-CSF we established that daily subcutaneous bolus doses of 3 to 15μg/kg/day gave optimal elevations of white cell levels (Lieschke et al., 1988). Higher doses were above the maximum tolerated dose (Lieschke et al., 1988). We are now using subcutaneously administered GM-CSF in patients with small cell lung cancer to abrogate the neutropenia caused by CBDCA and VP16 (Bishop et al., 1987). Patients receive 100mg/m^2 IV of CBDCA and 120mg/m^2 IV of VP 16 on Day 1 to 3. GM-CSF is administered to groups of 5 patients for varying intervals from day 4 to 11, 4 to 18 and 4 to 25. We have analyzed the first cycle and compared it to an 82 patient historical control groups who received treatment in the same fashion without GM-CSF (Morstyn et al., 1989(c)). In the first cycle without GM-CSF neutropenia was seen in 25/82 and with GM-CSF in only 1/11. We conclude GM-CSF is a promising agent for preventing chemotherapy induced neutropenia.

ADVERSE OBSERVATIONS ASSOCIATED WITH SUBCUTANEOUSLY ADMINISTERED COLONY STIMULATING FACTORS

Both G and GM-CSF are relatively well tolerated at the doses used in our studies. Both G-CSF and GM-CSF cause bone pain usually preventable by paracetamol. There are several adverse effects associated with GM-CSF including a reaction to the first dose, rashes and fevers that we have not observed following G-CSF administration. Some of these effects may be mediated by the release of other cytokines such as TNF or IL-1 released by monocytes or neutrophils activated by GM-CSF. Table 1 contains a list of adverse observations made in our phase I, II and III studies. The reaction to the first dose of GM-CSF observed in our studies was characterized by flushing, dyspnoea, hypotension and lumbar pain. The reaction was more common in patients receiving a short intravenous infusion than subcutaneous GM-CSF. We observed that a fall in oxygen saturation occurred after only the first dose of a course of daily injections of GM-CSF. In contrast serial DLCO measurements performed on two patients demonstrated the DLCO falls transiently with each dose of GM-CSF administered. This fall was no greater with the first dose than with subsequent doses however hypoxia only occurred with the first dose. We speculate that a fall in DLCO may be associated with the transient fall in neutrophils, monocytes and eosinophils that occurs immediately after each dose of GM-CSF is administered[10] whereas hypoxia may be due to vasodilation and to a physiological arterio-venous shunt that may occur after only the first dose of a daily course of injections. As yet we do not know what the mediator of the vasodilation reaction is and more studies are underway.

TABLE 1. Adverse observations associated with colony stimulating factor administration

Severity of observation	GM-CSF	G-CSF
Low grade	Bone pain Lethargy Rash First dose effect (hypoxia, hypotension) Fever Thrombophlebitis	Bone pain Abnormal taste
	Elevated AP, γGT Hypoalbuminaemia	Elevated AP & LDH Elevated urate
Dose-limiting	Pericarditis Fluid retention	None identified
Contraindications	Autoimmune thrombocytopenia	None identified

CONCLUSIONS AND FUTURE DIRECTIONS

Both G-CSF and GM-CSF appear effective at preventing the neutropenia associated with standard chemotherapy. GM-CSF has a broader action in that it stimulates monocytes to a greater extent than G-CSF and also eosinophils. It also seems to be associated with a broader range of adverse observations at the doses tested than G-CSF, however studies with G-CSF are at an earlier stage and fewer patients have been tested. It is possible that some of the adverse effects associated with GM-CSF are due to the release of other cytokines by activated monocytes. It is also possible that the dose chosen in some of the phase II studies is too high. We chose 15µg/Kg/day SC of GM-CSF in the small cell lung cancer study which may have been unnecessarily high since 3µg/Kg/day SC gave similar fold elevations in neutrophils. It is possible that lower doses will have similar efficacy with less adverse observations. There are also no comparisons of the efficacy of G-CSF and GM-CSF in preventing infections. In the future we may use mixtures of cytokines, for example, G-CSF and GM-CSF together particularly if it can be shown that there are advantages in producing effects additional to elevations in just neutrophils alone. If elevations in neutrophils are all that is required G-CSF would seem to be very effective as a single agent.

A major hope is that strategies will also be developed to prevent thrombocytopenia and mucositis and we will be able to escalate the dose intensity of chemotherapy and achieve better anti-cancer effects. It already seems clear that G-CSF has a major impact in reducing the morbidity and cost associated with autologous marrow transplantation and will become widely used for this indication. We now know the optimal doses and routes of administration of G-CSF and GM-CSF and the challenge is to integrate these agents in an optimal way into cancer therapy.

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THE BIOLOGICAL BASIS FOR INTERLEUKIN-2 ANTICANCER THERAPY

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The biologic activity of soluble immune modulators was inferred from functional assays almost 25 years ago (Bloom and Bennett, 1966). With the recent availability of monoclonal antibodies and the impact of recombinant DNA techniques the molecular basis for such activity has begun to emerge. We can now begin to understand how the agents, their receptors and effector cells interact to produce antitumor effects. Recent clinical studies have affirmed the therapeutic potential for these agents.

INTERLEUKIN-2

Evidence of a growth promoter for T-lymphocytes was first reported by Morgan and colleagues in 1976. Originally known as T-cell growth factor, further definition as an inter-leukocyte messenger resulted in the designation, interleukin-2 (IL-2). Physicochemical studies of highly purified IL-2 revealed a molecular weight of about 15,500 Daltons, with some variation ascribed to glycosylation, and a pI of 8.2 after removal of sialic acid residues (Robb and Smith, 1981). The tertiary core structure, dictated by amino acid sequence and a disulfide bond between residues 58 and 105, is required for biological activity (Yamada et al., 1987). Full activity is retained with minor variation in amino acid sequence. For example serine has been substituted for cysteine at position 125 in the Cetus Corporation recombinant IL-2 (Doyle et al., 1985). This interesting example of genetic engineering avoids unwanted alternate disulfide bridging. Glycosylation is clearly not required for activity as the commonly used recombinant products are not glycosylated.

In evaluating clinical activity it is important that the various units of activity are only now becoming uniform. Clinical reports to date using Cetus Corp. IL-2 use units where 1 Cetus unit = 6 international units. Similarly Hoffman-LaRoche used BRMP units which are equivalent to 2.6 international units (personal communication Cetus Corp.).

IL-2 RECEPTOR

The complete high affinity ($Kd=10^{-11}M$) receptor consists of a heterodimer with a larger unit of 75 kD (p75) non-covalently linked to a smaller unit of 55 kD (p55) (Tsudo et al., 1986). It is the p55 unit that was identified by anti-Tac antibodies. The p75 component alone is sufficient for cellular activation and can bind IL-2, although at much reduced binding affinity ($Kd=10^{-9}M$) (Tsudo et al., 1986). It now appears that p75 can be expressed constitutively on non-MHC restricted cytotoxic cells, B cells, and possibly monocytes (Tsudo et al., 1987; Nakanishi et al., 1984; Malkovsky et al., 1987). This would provide a basis for direct stimulation at relatively high IL-2 concentrations. Cellular expression of the complete high affinity heterodimer is normally restricted to specific antigen or mitogen stimulated T-cells. This suggests that the function of p55 is to allow high affinity binding for activated T-cells and thus act as a regulator of receptor activity.

LIGAND-RECEPTOR ACTIVITY

T-cell activation is initiated by specific MHC-restricted binding to the T-cell receptor. The activation signal is followed by appearance of both the high affinity IL-2 receptor and production of IL-2, possibly through concurrent elaboration of γ-IFN, (Wano et al., 1988).

Similar autocrine mechanisms may be a common feature of cytokine activation. Since exogenous IL-2 is required to maintain activated T-cells in culture there must be a related mechanism for down regulation, possibly through p55 (Gillis et al., 1978).

The exact mechanism for IL-2 receptor induction of activated T-cell proliferation is not clear. High affinity IL-2 receptors are internalized (Weissman et al., 1986). Subsequent activation of proto-oncogenes such as c-myc, c-myb and pim-1 suggests a proliferation signal similar to that induced by platelet derived growth factor and epidermal growth factor (Reed et al., 1988).

CELLULAR EFFECTORS

Activated T-Cells

Most of the data on mechanisms of action of IL-2 comes from observations of T-cells. In this case by inducing proliferation IL-2 serves as an amplification mechanism for the antigen specific T-cell receptor. In terms of exogenous IL-2 administration this means that relatively modest doses should be sufficient to interact with the high affinity IL-2 receptors of previously primed T-cells. For this approach to be clinically useful the neoplasm must express tumor associated antigen which has already been recognized by T-cells at the time of IL-2 administration. The possibility that T-suppressors may also be activated provided the rationale for concurrent use of cyclophosphamide in some treatment programs (Rosenberg et al., 1986).

Non-MHC Restricted Killer Cells

Natural killer (NK) and lymphokine activated killer (LAK) cells are similar in that they are morphologically large granular lymphocytes, can attack a broad range of tumor and virus infected cells and are stimulated by IL-2. The main difference is functional. LAK cells will attack certain cell lines not affected by NK, such as the Daudi cell line and many fresh tumor cells. Although both are usually characterized phenotypically as $CD3^-$, Leu 11^+ or Leu 16^+, some LAK cells may express the CD3 marker (Ochoa et al., 1989). Direct activation by LAK is probably through constitutively expressed p75 receptors, and this may explain why relatively high exogenous IL-2 doses are required for LAK activation. It is not clear how the cellular mechanism of IL-2 activation in non-MHC restricted killer cells relates to IL-2 induced proliferation in specifically activated T-cells.

Attention has focussed on LAK cells as the probable mediator of clinical anti-tumor activity in humans. Induction of peripheral LAK activity appears to be a necessary antecedent in responding patients, but is not sufficient to predict tumor response. Further, infused LAK cells do not track well to tumor sites and responding tumors are not necessarily infiltrated by large numbers of LAK cells (Cohen et al., 1987).

Tumor Infiltrating Lymphocytes (TIL)

Lymphocytes extracted from tumor tissue (TIL) may be superior to LAK for adoptive immunotherapy. This was clearly the case in an animal model (Rosenberg et al., 1986). TIL usually include a mixed population of non-MHC restricted and MHC restricted cytotoxic cells (Rabinowich et al., 1987; Heo et al., 1987). Incubation of such cells with IL-2 was initially reported to result in a 50-100 fold increase in cytotoxicity compared to IL-2 exposed peripheral blood leukocytes (Rosenberg et al., 1986). Others feel increased overall cytotoxicity of TIL is a reflection of enhanced proliferation and that individual TIL are no more cytotoxic than LAK (Heo et al., 1987; Belldegrun et al., 1988). The relative contribution of MHC restricted and non-restricted effectors appears to vary widely depending on tumor type, individual variation, and duration of culture (Heo et al., 1987; Topalian et al., 1988; Knuth et al., 1984; Itoh et al., 1986).

An additional potential advantage of adoptive immunotherapy with TIL compared with LAK is that in some hands TIL may have considerably improved capacity to track to tumor sites (Fisher et al., 1989). Others have not found specific tracking, perhaps because of differences in tumor type, timing or variation in technique (Kradin et al., 1987).

CYTOKINE INTERACTIONS

Antitumor effects of IL-2 are at least in part dependent on interacting cytokines. IL-1 enhances antigen specific T-cell activation (Dinarello et al., 1986). Not only does the γ-IFN produced by activated T-cells induce IL-2 production, but it also stimulates macrophages (Silver et al., 1986). Other cytokines produced by activated T-cells include tumor necrosis factor and a B-cell activation factor (Fauci et al., 1987). With the rapidly expanding list of cytokines and more detailed knowledge of their pleotropic biologic activity the possibilities of interaction have become exceedingly complex. Although much is to be learned it is clear that both the antitumor activity and toxicity of infused IL-2 may in part be mediated by other cytokines.

IL-2 IN ANIMAL TUMOR MODELS

The most extensive pre-clinical studies have been conducted by Rosenberg and associates and these have been reviewed in detail (Rosenberg, 1987a). The adoptive immunotherapy approach to IL-2 evolved from these studies. Results were similar in mouse models for immunogenic and non-immunogenic methylcholanthrine induced sarcomas, melanomas and adenocarcinomas. Both lung and hepatic metastases were used as end points. In these models therapy began about 3 days after tumor injection and metastases were counted in the second week. While there are a number of ways such animal models are not analagous to well established spontaneous human tumors, several interesting observations were made. Compared with controls a significant reduction of metastases was seen with IL-2 and there was a dose response. However, the high IL-2 doses required for significant inhibition of metastases (up to 90,000 units/day/mouse) would not be tolerated in humans. Effective _ex vivo_

LAK generation required at least 3 days incubation with IL-2. The combination of these LAK with IL-2 was much more active than either agent alone and allowed effective treatment with much reduced IL-2 doses.

CLINICAL STUDIES

Tumor Response

Guided by his animal and phase I clinical studies of IL-2 as a single agent, Rosenberg evolved a formula for treatment (Rosenberg et al., 1985). IL-2 was given by iv bolus three times per day in doses up to 100,000 u/kg.

To generate cells for leukophoresis patients began a 5 day course of IL-2 at up to 100,000 u/kg three times per day. After allowing 3 days for rebound lymphocytosis daily leukophoresis was performed and harvested leukocytes were then exposed to IL-2 *in vitro* for 5 days. These were then sequentially reinfused during another course of IL-2. In some patients treatments were repeated at 3 week intervals.

This experience has subsequently been updated and compared with patients who received IL-2 alone by a variety of doses and schedules (Rosenberg et al., 1987b). Of the 106 evaluable patients treated with IL-2 + LAK there were 8 CR and 15 PR for a total of 22% CR + PR. Since it had previously been felt that IL-2 alone would be ineffective, it is interesting that responses were also seen among those treated with IL-2 alone. Of the 46 evaluable patients so treated there were 1 CR and 5 PR.

The tumors of greatest interest have been renal carcinoma and malignant melanoma. Results of more recent studies of these sites have included an overall response rate for malignant melanoma of 21% of 48 patients and for renal cell carcinoma, 31% of 54 patients (Rosenberg et al., 1988a; Rosenberg et al., 1988b). While the melanoma experience has been generally in keeping with others, another group achieved responses in only 14% of 35 renal cell carcinoma patients (Dutcher et al., 1987; Fisher et al., 1988). Such differences in response rate might best be explained by patient differences and the relatively small sample size. In either event there is no doubt that the treatment induces clear responses in a significant number of patients. Unfortunately, there has also been significant toxicity.

Toxicity

Relatively non-specific symptoms of fever, chills, malaise, and GI upset are common, but can frequently be managed with symptomatic medication. Anemia and thrombocytopenia, while common, have not been a serious symptomatic problem. Of much greater concern are the problems related to increased capillary permeability, the so-called "capillary leak" syndrome. This acute shift of fluids to the extravascular compartment has been responsible for serious toxicity.

In a series of 127 patients this included a greater than 5% weight gain in 59%, hypotension requiring support of pressor agents in 70%, respiratory distress in 18% (requiring intubation in 8%), elevated creatinine in 80%, myocardial infarction in 1 patient and death in 1 patient (Rosenberg et al., 1987b). Such patients must remain under close observation in hospital at least, others have required intensive care. Comparable toxicity has been seen in patients receiving the same high doses of IL-2 without added LAK infusions (Rosenberg et al., 1987b). Clearly, high dose IL-2 is both too impractical and too toxic for routine use. Intensive efforts are underway to reduce toxicity while maintaining or improving response.

EVOLVING APPROACHES TO TREATMENT

Effector Cell Enhancement

One approach has been to try and select better effectors for adoptive immunotherapy. As discussed above this could be the case for tumor infiltrating lymphocytes (TIL). While somewhat controversial, it is possible that TIL have improved cytotoxicity, specificity, and ability to track to tumor sites. In a recently reported study significant responses were seen in malignant melanoma (Rosenberg et al., 1988b).

The actual response rate is difficult to specify since some patients were rendered ineligible for TIL therapy when disease progressed during the period of TIL growth in tissue culture. This tended to eliminate poor risk patients from study. The response rate could be viewed as between 37% of 30 patients or 60% of 15 patients. IL-2 was given in the same high doses described above, but toxicity was decreased because treatment duration was shorter (Rosenberg et al., 1988b). The results of other TIL studies in progress will be of great interest.

The inconsistency in TIL results may be related to the heterogeneous cell population being studied. The best effectors may be relatively evanescent in tissue culture. With prolonged culture LAK cells with only modest cytotoxicity come to predominate (Heo et al., 1987; Belldegrun et al., 1988). Improved selection and culture techniques may be needed for reliable results.

Combination Treatment

A variety of approaches have been suggested that could enhance IL-2 antitumor effects and thus permit more clinically acceptable IL-2 doses. There is evidence that IL-1 or TNF can enhance IL-2 induced immune effectors (Brunda et al., 1986; Krigel et al., 1988; Crump et al., 1989; Owen-Schaub et al., 1988). Another approach would be to use agents that might block the negative regulatory activity of suppressor cells which can be detected at tumor sites and may be stimulated by exogenous IL-2. Strategies which appear to work in clinical models include the use of cyclophosphamide, to which suppressor cells are relatively sensitive, or indomethacin, which could interrupt the suppressor effects of prostaglandin E_2 (Rosenberg et al., 1986; Lala and Parhar, 1988; Silagi and Schaefer, 1986). Larger phase I or phase II studies will be required to confirm efficacy in humans.

Combination therapy with antineoplastic chemotherapy has also been evaluated. Not only might such combinations be synergistic, but the eradication of chemotherapy resistant cell lines could have a dramatic impact on cancer survival (Goldie and Coldman, 1979). We have been evaluating the combination of dimethyl triazeno imidazole carboxamide (DTIC) chemotherapy and relatively low doses of IL-2 in malignant melanoma patients. DTIC was given in a standard regimen of 250 mg/m^2. The IL-2 administration schedule was selected to reduce the acute toxic effects of IL-2. Our results for the first 8 patients have been interesting in this regard. While the acute toxicity has not been a problem, more chronic effects of IL-2 administration became dose limiting. These include marked fatigue and malaise, erythroderma with desquamation in some patients, and arthralgia/myalgia not controlled with indomethacin. Our experience suggests that chronic toxicity will be a problem with prolonged IL-2 exposure. It is not yet possible to determine if the character of antitumor activity is any different from what would be expected for DTIC as a single agent.

Directed Administration

It may be possible to improve local cytotoxic effects while reducing systemic toxicity by directing IL-2 and/or LAK cells to organ or tumor sites within the body. Trials are underway looking at intratumoral, intraperitoneal, perilymphatic and intra-arterial tumor directed therapy (Chapman et al., 1988; Ottow et al., 1987; Cortesina et al., 1988; Yoshida et al., 1988; Ishikawa et al., 1988; Morita et al., 1987).

Our own approach to directed administration has been to deliver IL-2 by splenic artery perfusion (Klasa et al., 1988). Anatomic and physiologic features making this strategy attractive include: the spleen is a large reservoir of lymphocytes (about 25% of the total lymphocyte pool), the spleen is an organ receiving only about 5% of the cardiac output, and IL-2 has a relatively short half-life. It should thus be possible to deliver relatively high IL-2 doses to splenic lymphocytes with much reduced systemic exposure. In this study patients received from 1.5 to 4.0 x 10^4 Cetus units/kg daily x 5 days by intrasplenic perfusion. Treatments were repeated at 3 wk intervals depending on response. Among the 20 patients treated to date systemic toxicity has been clearly less marked than expected for high dose systemic treatment (Klasa and Silver, 1989b). Although significant hypotension has been seen in 8 patients, this has not required pressor agents. Fluid retention >5% body weight has only been seen in 2 patients. On the other hand, there has been a marked augmentation of peripheral NK and LAK activity (Klasa and Silver, 1989a). Further, unlike systemic IL-2 with intrasplenic administration cellular activation is retained in the week following IL-2 administration. Although we have seen disappearance of metastatic lesions following treatment, it is not yet clear how character of response compares with other IL-2 regimens.

Prospectives

Current clinical experience has demonstrated that IL-2 therapy can produce significant responses. Il-2 will only become non-experimental standard therapy when these antitumor effects are achieved with less toxicity. This could well be possible thanks to an improving understanding of interacting cytokines, combination treatment with chemotherapeutic agents or other biologicals, and advances in directed therapy. In keeping with other biological agents more marked therapeutic benefit would be expected with sustained treatment in patients with minimal tumor burden.

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MECHANISMS OF ACTION OF INTERFERON

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As biological response modifiers, interferons have a broad range of pleiotropic biological activities. By definition, each of the interferons is anti-viral, and capable of inhibiting viral replication in physiologically minute quantities. The interferons also function with dramatic immunomodulatory activity. This includes their ability to augment natural killer cell function as well as to up-regulate cytotoxic T cell function. Additional data from our own laboratory have demonstrated the ability of alpha interferon to influence immunoglobulin production by B cells, indirectly by increasing helper T cell activity. Lymphocytes and tumor cells are also affected immunologically by increased expression of class I and class II histocompatibility antigens that are associated with a variety of alterations in their protein synthetic pathways.

However, perhaps the most significant activity of the interferons is as negative growth regulators. In this capacity they demonstrate anti-proliferative activity toward a wide variety of both normal and malignant cells in vitro, although only a few tumors are usefully treated in the clinic.

Interferons have been demonstrated to inhibit the expression of at least three oncogenes (myc, ras, and src), and they appear to play a significant role in the differentiation of both myeloid and lymphoid cells. Perhaps most importantly, interferon production is induced by many agents that stimulate growth, resulting in growth inhibition. Thus, interferon acts to close the negative feedback loop initiated by positive growth factors. It may be that the anti-viral, anti-proliferative, and anti-tumor effects of the interferons are all related to the growth inhibition that they induce.

In clinical trials in chronic myelocytic leukemia, for example, inhibition of replication of malignant myeloid stem cells leads to reductions in all formed elements in the myeloid series, a slow but continuing reduction in the proportion of Philadelphia chromosome positive metaphases in patients' bone marrow during therapy and a concomitant reduction in cells expressing the 9:22 translocation by Southern blot analysis utilizing BCR probes. Efforts to understand the mechanism of negative growth regulation inhibiting tumor stem cell self-renewal are currently focusing on interferon receptor binding and intracellular turnover. Methodologies to identify the type I and II receptors have been developed, the type II receptor has been cloned, and efforts are currently underway in our own laboratory to clone the typeI receptor. A better understanding of the mechanisms of negative and positive growth regulation by each of the interleukins should provide improved understanding of their clinical activity in human disease.

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THE GROWTH KINETICS OF CLONOGENIC TUMOR CELLS THAT SURVIVE RADIATION THERAPY

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INTRODUCTION

The question of whether radiotherapy or therapy by any other modality causes significant changes in the cell population kinetics of tumors remains a controversial one. As reviewed by Steel (1977), there is evidence both for shortening and lengthening of the cell cycle and its component phases. One investigation that is perhaps the most directly applicable to this controversy is that of Hermens and Barendsen (1969) who reported that the surviving clonogenic cells of the R-1 rhabdomyosarcoma in the rat grew more rapidly after the tumor was exposed to 2000 rads of X rays. That finding is consistent with much recent experience in the clinic. Evidence continues to accumulate to support the inference that tumors may undergo accelerated repopulation after exposure to curative conventionally fractionated radiotherapy -- and, especially, during the course of such a regimen (Kummermehr, et al., 1986; Thames & Hendry, 1987). Although one cannot question such observations, their interpretation is debatable; and insofar as the interpretation and indeed the identification of the responsible mechanisms may well determine the choice of means to overcome or otherwise modify this effect, it is highly desirable to examine the mechanisms that might account for it. Briefly, there are three main candidates for a responsible mechanism: shortening of the cell cycle, increasing the growth fraction, and reducing cell loss. If accelerated repopulation results from changes in the cell kinetic parameters of cycling cells, for example, one might seriously consider a limited number of approaches that could be used to modify the effect or even to capitalize upon it in the design of more effective therapies. If the accelerated repopulation were attributable to changes in the growth fraction, however, a different set of approaches would be in order.

In examining such a question, one should first examine the methodology that can be used to determine cell kinetic changes. Is it legitimate, for example, to use classical methods to determine the kinetic parameters of cell populations that are perturbed by therapy? The approach that has been used principally in determining such parameters is the labeled mitosis method. However, we must be reminded that this method was developed for, and should be restricted to, steady state populations. This certainly excludes cells perturbed by a wide range of therapeutic modalities, so we may well wonder about the validity of cell cycle parameters that are obtained by this methodology in, say, irradiated tumors.

We have developed a completely different approach to examining the kinetics and growth rate of a limited number of experimental tumors, and have applied this method to the question of accelerated repopulation. In curative cancer therapy the most relevant cell in any tumor is clearly the surviving clonogenic tumor cell, i.e. the tumorigenic cell. In normal tissues, the comparably relevant cell would be the intact surviving stem cell.

A number of experimental animal tumors have been developed that grow equally well in vitro as in vivo. In the case of the EMT6 tumor, the TD_{50} is but a few cells and the plating efficiency of cell suspensions in vitro prepared from solid tumors growing in vivo usually ranges between 30 and 50%. After but a single passage in vitro, the P.E. rises to close to 100% because the nonclonogenic tissue cells that "contaminate" every fresh tumor cell suspension will have been eliminated or far outnumbered. Allowing single cells to grow into macrocolonies under the most favorable conditions in vitro is a convenient means of distinguishing clonogenic surviving tumor cells from non-survivors. When studying radiation effects, this is essential as one can easily be misled into attributing effects on cells destined to die to those that still possess full clonogenic capability (Hurwitz & Tolmach,1969; Hopwood

& Tolmach, 1979). Therefore, examining the kinetic parameters of such colony-forming cells and their progeny, constitutes an effective means of discriminating the truly relevant survivors in the clonogenic subpopulation from the other cells present in an intact tumor.

Measuring the intermitotic times of surviving clonogens by the application of time lapse cinemicrographic methods provides a simple and direct means of obtaining cell kinetic parameters, both of the cells that were present at the time of treatment and also their progeny through several post-treatment generations. To be sure, growth rates may not be the same for cells grown under the artificial conditions that obtain in vitro as contrasted with the conditions that exist in vivo, i.e. the conditions that would be experienced by cells allowed to remain in situ in the host; but this is an inherent disadvantage that simply cannot be avoided. This report presents data that relate directly to the growth potential of surviving clonogens; and because the methodology is novel and has not yet been explained in detail elsewhere, it will be presented at some length here.

MATERIALS AND METHODS

Biological materials. All of the data reported herein were obtained with the cells of EMT6 tumors (Rockwell, et al., 1972) propagated and treated in syngeneic BALB/c mice. Tumors were grown from intradermal inocula of 10^5 cells and were irradiated when they reached approximately 6-8 mm in average diameter. Shortly after the completion of the irradiation treatment, tumors were excised and disaggregated into single cell suspensions with the aid of trypsin (0.05% in Hanks' balanced salt solution). Cell counts were obtained using phase contrast optics and a conventional phase contrast hemacytometer. All large tumor cells that excluded trypan blue dye were counted, and the suspensions were adjusted to concentrations so that approximately 100 surviving macrocolony-forming cells could be inoculated into single chamber Lab Tek culture slides, the tissue culture vessel that was used throughout this work. The final dilution of the freshly counted cells was made into prewarmed complete culture medium (Waymouth's) containing 15% fetal calf serum in culture slides.

The slides were loaded into a specially constructed mini-incubator which is clamped on the programmable scanning stage of an inverted microscope. Usually, 6-10 hours are allowed to elapse in order that the cells can settle through the culture medium to attach to the glass surface at the bottom. Then an observer scans each slide chamber and maps the position of each of a desired number of tumor cells. Earlier mapping was done only in X and Y coordinates; but we have recently added a stepper motor to the microscope's fine adjustment so that we can now map in X, Y, and Z. All observation is done at low magnification (4x objective lens) using modulation contrast optics (Hoffman, 1977). In each experiment, we customarily map the positions of at least about 30, but no more than 100, cell positions. Each set of coordinates is stored in the memory of a microcomputer, and after the mapping is completed the computer is programmed to direct the stage and the fine adjustment to revisit each of the cell positions in rapid succession at regular intervals. As the stage arrives at a given position, the microscope lamp is turned on automatically, the light intensity is read by an exposure meter, and a picture is taken with a 16-mm Bolex cine camera. In all experiments, we have used a 20 min interpicture, i.e. time-lapse, interval and have recorded images for as long as 2-1/2 weeks. Currently, this apparatus limits us to the use of 800 feet of film, which is 32,000 frames. At the end of a typical period of photographed cell growth, we have a continuous length of primary film bearing images in successive frames that are not in the correct sequence for projection as a motion picture. The frames must therefore be rearranged; and this is done by rephotographing them onto a secondary film, using a special film projector that can be computer operated and using our 16-mm Bolex camera to rephotograph the images.

For analysis, secondary films are televised and viewed frame by frame on a video monitor. A number of events are scored, including every division, cell death, cell fusion, etc. All observations are stored in the memory of a microcomputer (IBM PC/XT); and we have been able to trace successive divisions within colonies for up to 9 generations. It may be seen that this system permits us to distinguish true clonogenic survivors from those formerly clonogenic single cells that are limited to perhaps 2-4 postirradiation cell divisions before death or the cessation of further division.

Irradiation conditions. All tumors were irradiated together with the rest of the mouse in a whole body setup. The X-ray machine was operated at 250 kVp with 0.35 mm Cu filtration, giving a HVL of 1.0 mm Cu. The dose rate varied between 0.8 and 1.2 Gy/min. The present report is based upon the effects of a total dose to the tumors of either 3 or 6 Gy given either as a single dose (previously reported: Kallman, 1988), 3 x 2 Gy (a fractional dose of 2 Gy given on 3 successive days) and 5 x 1.2 Gy (a fractional dose of 1.2 Gy given at 9 a.m. and 3-4 p.m. on 2 successive days and 9 a.m. on the 3rd day).

RESULTS AND DISCUSSION

On the occasion of this symposium, this presentation consisted largely of a videotape, because that is the optimal means of illustrating the kinds of motion pictures that it is possible to produce in order to answer some of the specific key questions in cancer therapy posed in this presentation. Also, insofar as our experimental approach is a novel one, this symposium is an ideal means of informing others of the applicability of this methodology. Because of the limitations of the printed page, this report will give only some final data relevant to the question of accelerated repopulation.

The interdivisional times summarized in Tables 1 and 2 constitute a definitive body of data that support a number of conclusions. First, the growth of these EMT6 clonogenic cells provides an excellent example of the classical lag phase that is seen in virtually any kind of cell (bacterial to mammalian) that is explanted for in vitro culture. This is true for both unirradiated and irradiated cells, nor does it appear that irradiation in this case caused any significant increase in the duration of the lag phase. Second, although the mean interdivisional times show fluctuations after the lag phase, there is no systematic or significant difference in mean interdivisional time throughout the course of the next 8 successive generations in colonies arising from unirradiated or irradiated progenitor cells. The final (8th generation)interdivisional times for the unirradiated controls and the cells exposed to 6 Gy are remarkably short, but this is an artifact that arises from the inadvertent selection of cohorts of cells that are rapid dividers, simply because they could not otherwise be observed in the limited time over which filming was done in those experiments. As shown in the lower part of the Table, however, it makes very little difference whether that last generation is or is not included in the final data analysis. Third, these data demonstrate incontrovertably that EMT6 tumor cells that survive exposure to 3 or 6 Gy *in vivo* provide no evidence for a shortening of the cell cycle time: the corrected average interdivisional time for cells whose progenitors were exposed to 3 Gy was at least 5 hours longer than controls. Cells surviving double that radiation dose also had longer interdivisional times, but for presently unaccountable reasons the mean was shorter than that found for the lower single dose of 3 Gy.

Table 1. Interdivisional time statistics of dividing cells in colonies grown from unirradiated EMT6 tumor cells.

Generation	No. cells/ sequences	$\overline{T}_i$ ±	s_{T_i}
0	25/25	35.20	16.50
1	43/22	16.00	5.11
2	83/22	11.94	3.50
3	164/22	11.99	3.97
4	312/21	13.46	5.57
5	532/21	13.37	5.47
6	640/20	13.21	5.24
7	408/19	10.94	5.77
8	109/15	8.32	6.65
All	2316	12.81	3.57
All but 0	2291	12.56	3.53
All but 0 & last	2182	12.77	3.56

In column 2, the sequences (denominator) are the colonies whose growth could be traced in time lapse films; and the numbers of cells (numerator) are the total numbers that divided and could be traced in the generation specified in column 1.

Table 2. Interdivisional time statistics of dividing cells in colonies grown from EMT6 tumor cells irradiated with single doses of 3 or 6 Gy.

	3 Gy			6 Gy		
Generation	No. cells/ sequences	$\overline{T}_i$ ±	s_{T_i}	No. cells/ sequences	$\overline{T}_i$ ±	s_{T_i}
0	46/46	38.20	13.15	55/55	36.05	10.04
1	75/42	14.56	12.21	98/52	16.67	8.25
2	111/38	17.21	15.57	165/49	16.02	10.26
3	160/36	20.18	11.20	259.48	17.01	9.91
4	215/33	20.47	12.26	371/42	17.05	10.12
5	232/29	18.18	9.74	479/38	15.26	7.99
6	206/23	16.42	9.73	454/34	12.91	6.97
7	119/17	14.87	10.27	300/26	10.83	6.61
8	52/9	15.04	9.24	160/21	8.90	6.03
All	1216	18.54	12.15	2341	14.88	9.35
All but 0	1170	17.76	11.44	2286	14.37	8.72
All but 0 & last	1118	17.89	11.52	2126	14.78	8.75

See Table 1 for footnotes.

Over the last few years especially, there has been increasing interest in the use of hyperfractionated radiotherapeutic regimens to compensate for anticipated accelerated repopulation (Thames, et al., 1983; Thames & Hendry, 1987; Withers, 1988). Indeed, clinical trials have been mounted to compare the therapeutic efficacy of hyperfractionated radiation therapy *versus* conventional dose fractionation (usually approximately 2 Gy/day for 4-6 weeks); and preliminary results from such trials have been most encouraging. Common features are the use of 2 or 3 radiation dose fractions/day, with fraction sizes of 1.2-1.8 Gy (i.e., greater than 1 and less than 2 Gy/fraction), and with fractions given no less than 6 hours apart. Even though such hyperfractionation regimens are designed not so much to prevent accelerated repopulation as to compensate for it, it is certainly conceivable and not illogical to predict that tumor cell repopulation might not be accelerated under such novel conditions. It was for this reason that we have extended our time lapse experiments into testing whether the mode of dose fractionation affects the rate of cell production in irradiated tumors.

We have set out to compare the interdivisional times of clonogenic surviving EMT6 tumor cells after exposure _in vivo_ to a single dose of 6 Gy _vs_. 3 doses of 2 Gy given daily (conventional fractionation) _vs_. 5 doses of 1.2 Gy given mostly twice a day (hyperfractionation). Unfortunately, these experiments have not been completed, but it is possible to detect certain trends in the limited data presently available (Table 3).

Table 3. Interdivisional time statistics of dividing cells in colonies grown from EMT6 tumor cells irradiated with 6 Gy in fractionated courses.

	3 x 1.2 Gy			5 x 1.2 Gy		
Generation	No. cells/ Sequences	$\overline{T}_i$ ±	s_{T_i}	No. cells/ sequences	$\overline{T}_i$ ±	s_{T_i}
0	30/30	42.64	19.00	25/25	50.61	19.72
1	36/20	20.84	11.05	12/9	23.75	8.62
2	33/14	15.92	6.04	7/4	18.48	6.06
3	35/8	12.35	4.11	1/1	26.00	
4	42/6	14.94	7.36			
5	15/3	13.53	6.57			
All	191	19.99	9.77	45	37.90	21.23
All but 0	161	15,77	8.03	20	22.02	8.65

See Table 1 for footnotes.

Whereas the data of Tables 1 and 2 relating to single doses and controls are referable to appreciable numbers of cell colonies of which probably the majority are true clonogenic survivors, this is hardly the case in Table 3.

It is reasonable to generalize that clonogenic cells whose descendants in the third or later postirradiation generation continue to divide may be regarded as reproductive survivors because the majority of nonsurvivors die at their first or second, or even third, attempted postirradiation mitosis (Hurwitz & Tolmach, 1969; Hopwood & Tolmach, 1979; Thompson & Suit, 1969). For example, as seen in Table 2 we were able to trace the reproductive behavior of 55 single cells that divided once after 6 Gy. Of the 110 daughter cells that resulted from that division, 12 could not be followed beyond the first division; the 98 that could be traced divided a second time at a mean of 16.67 hours after the first division; and 3 of the original 55 colonies aborted after they had reached the 2-cells stage. It may be seen that 3 more aborted after the 3-4-cells stage and colony abortions continued up to the 8th generation. This latter generalization is deceptive, however, because one's inability to obtain interdivisional times statistics for every colony is not always attributable to colony abortions: occasionally, cells will be lost to observation by their moving out of the photographic field either permanently or even temporarily. Also, the decline in the number of sequences scored in later generations, e.g. the 6th, 7th, or 8th, especially, also occurs simply because as colonies get larger it becomes increasingly more difficult to follow all of their cells; if the original progenitor cell was not centered in its field, later generation progeny will invariably migrate outside of the field and be lost to quantitative tracing. Also, as colonies come to contain greater and greater numbers of cells and become more and more compact, it becomes distressingly difficult for an observer to distinguish individual cells and thereby obtain an accurate record of cell divisions. These and other factors force us to score 7th and later generation cell divisions in increasingly fewer surviving colonies.

Nonetheless, the data relating to the duration of interdivisional time in the survivors of single doses of 6 Gy are not only statistically adequate but radiobiologically representative. This is not the case, however, for the experiments represented in Table 3. Of the 30 cells that underwent a first division after 3 x 2 Gy, for example, fewer than 8 and most likely only 3 were probably true radiation survivors. The columns of data under 5 x 1.2 Gy tell us even less about the kinetic behavior of true survivors, as only 1 cell was observed to undergo a 3rd postirradiation division, which occurred 26 hours after its birth in the 2nd generation. In spite of these limitations, the data that are tabulated give no suggestion that either fractionation or hyperfractionation might cause a shortening of the cell cycle of surviving clonogens; but it is obvious that many more data must be accumulated before any such conclusion could be stated with confidence.

The data presented above constitute strong evidence that the rate at which tumorigenic, i.e. clonogenic, tumor cells divide or progress through their generation cycles is certainly not shortened by exposure of their progenitors to X rays. On the contrary, if cell cycle time is changed at all, it appears to be lengthened. Even though it contrasts with some reports in the literature (Brown & Berry, 1969; Lord, 1986). this conclusion has been confirmed by the experiments of Ang et al. (1988) who have used a very different approach and a different mouse tumor. Because cell cycle time is prolonged, then, it follows that accelerated repopulation must result either from changes in the growth fraction or in the rate of cell loss in irradiated tumors. It is not illogical to propose that noncurative therapy may indeed stimulate the recruitment of noncycling clonogenic tumor cells, thereby increasing the effective growth fraction; and we among others are investigating this important question.

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MICROENVIRONMENTAL CHANGES IN IRRADIATED TUMORS

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INTRODUCTION

The environment surrounding a cell has a major impact on the characteristics and behavior of the cell and on the response of the cell to treatment with agents used in cancer therapy. Transient and persistant perfusion deficits lead to regional variations in the environment within solid tumors; the resulting microenvironmental heterogeneity produces regional and temporal variations in the sensitivity of the malignant cells to radiation and anticancer drugs. After treatment with a cytotoxic agent, cell death, tumor shrinkage, and stromal damage produce changes in the intratumor environment, which alter the response of the tumor to subsequent treatments. Microenvironmental heterogeneity produces variations in therapeutic responsiveness that will be superimposed upon any variability caused by the presence of multiple genetically-distinct subpopulations within the tumor (Heppner, 1984).

There are no simple genetic changes that can lead to the development of dramatic radioresistance analogous to the resistance observed for many drugs. Efforts to isolate radioresistant mutants from irradiated cell populations have generally proven fruitless; in fact most cell lines derived from heavily-irradiated populations are more radiosensitive than the parental lines (Sinclair, 1964; Suit, 1966; Ando et al., 1988). Thus, although the intrinsic radioresistance of the initial tumor cell population will be of great significance in determining the efficacy of radiotherapy, the development of a genetically radioresistant population during radiotherapy is not a problem. Environmental factors, in contrast, may be of great significance in modulating the response of the residual tumor to treatment.

TUMOR BLOOD FLOW AND TUMOR OXYGENATION

Radiobiologists have long been interested in tumor oxygenation, because molecular oxygen (O_2) is an extremely potent radiosensitizer. If O_2 is present during irradiation, this very electron-affinic molecule participates in and multiplies the chemical reactions leading to the production of cytotoxic lesions. Aerobic cells are about 3 times more sensitive to x-rays than are hypoxic cells. Very low levels of O_2 are required for radiosensitization, with essentially maximal sensitization attained at pO_2s below those found in normal venous blood. Most normal tissues therefore exhibit maximal, or near-maximal, aerobic radiosensitivity.

Tumors, in contrast, are supported by abnormal, deficient vascular beds, and include regions subject to transient and persistant deficits in blood flow. Thomlinson and Gray (1955) presented evidence that viable cells exist in a state of chronic hypoxia on the edges of avascular necrotic areas in corded tumors. In other solid tumors, regions of chronic hypoxia are scattered throughout the tumors in and near regions of necrosis, in areas where tumor cell growth has outpaced the growth of the vasculature, and in regions where pressure, vessel invasion, or thrombosis have led to chronic cessation of blood flow. Intermittent flow through individual blood vessels also leads to the development of transient hypoxia within solid tumors (Chaplin et al., 1987), with cells fluctuating between a well perfused (well oxygenated) state and an unperfused (hypoxic) state at regular or irregular intervals of minutes or hours.

Both transiently hypoxic and chronically hypoxic cells would be radioresistant. However, their physiologic characteristics and their sensitivities to other therapeutic agents may be quite distinct. For example, because the diffusion distances of glucose and many other nutrients in respiring tissue are similar to those of

O_2, chronically hypoxic cells will also experience chronic deficiencies in glucose and nutrients (Tannock, 1972). The regional extracellular pH may also be low, and the levels of lactate and other metabolic by-products may be high. These chronic environmental deficiencies can inhibit cell proliferation, with cells either forced into a G_0-like state or scattered throughout the cell cycle, but progressing through the cycle at a reduced rate or quiescent (Tannock, 1972; Allison et al., 1985). The proliferative state of the cells and the rate at which cells traverse the cell cycle both influence the response of the cells to cycle-dependent cytotoxic agents. Chronically hypoxic cells may also exhibit altered enzyme and protein profiles and/or enzyme activity patterns (e.g. the induction of glucose regulated proteins and other stress proteins, reduced levels of topoisomerase II, altered patterns of reductase activity) and altered cell membranes. These can influence the response of cells to drugs and radiation. As many of these changes develop only after several hours of exposure to environmental stress, the proliferative and physiologic characteristics of transiently hypoxic cells more closely resemble those of cells in well perfused areas.

Microenvironmental heterogeneity within solid tumors influences the efficiency of chemotherapy, as well as that of radiation. O_2 may participate directly in the chemical reactions leading to the cytotoxicity of some drugs (e.g. bleomycin) or alternatively may inhibit the metabolism of reductively-activated drugs to cytotoxic forms (e.g. mitomycin C, porfiromycin, misonidazole). Alterations in cell proliferation in hypoxia modulate the sensitivity of the cells to drugs having effects which vary with cell age, proliferative status, or proliferation rate. As described below, concommitant heterogeneity in pH and in distribution of active drug may also contibute to variability in the response of tumor cells to chemotherapy. Studies of solid tumors in animals show that microenvironmental heterogeneity does influence the efficacy of anticancer drugs. Adriamycin and nitrogen mustard, for example, are less effective against the hypoxic tumor cells than against the aerobic cells (Tannock, 1982; Hill and Bush, 1977). In contrast, misonidazole and porfiromycin are selectively toxic to hypoxic tumor cells in vivo (Kennedy, 1987; Rockwell et al., 1988). Microenvironmental heterogeneity therefore influences the response of tumors to chemotherapy, as well as radiation.

HYPOXIC FRACTIONS OF SOLID TUMORS

Solid tumors may contain relatively large numbers of viable, hypoxic tumor cells (Moulder and Rockwell, 1987). The proportion of viable hypoxic cells has been measured in several dozen transplanted solid rodent tumors, using techniques which examine the radiosensitivity of the clonogenic tumor cells (i.e. the cells which are capable of forming a clone, contributing to tumor growth, or causing a recurrence). The vast majority of macroscopic rodent tumors contain hypoxic cells; in most tumors 10-20% of the clonogenic cells are radiobiologically hypoxic. The heterogenous oxygenation revealed by the radiobiologic techniques has been confirmed in studies using O_2 microelectrodes, measurements of hemoglobin saturation and cytochrome reduction, NMR, and other techniques (Moulder and Rockwell, 1987; Vaupel et al., 1981; Kallinowski et al., 1988).

Hypoxic cells are not unique to rat and mouse tumors which have been passaged repeatedly (Moulder and Rockwell, 1987; Moulder et al., 1988). Early transplant-passage tumors and autochthonous tumors (tumors studied in the host of origin) have hypoxic fractions similar to those of serially-passaged tumors. Human tumors xenografted into nude mice exhibit a spectrum of hypoxic fractions similar to that of rodent tumors.

In many transplantable rodent tumor systems the hypoxic fraction increases with the tumor size. However, it is notable that even small (1-2 mm) tumor nodules and microscopic tumor deposits have been found to contain significant numbers of hypoxic cells. If this is also true in humans, hypoxia would influence the response of subclinical tumors, and even microscopic disease such as that remaining in involved margins after surgery.

Reoxygenation

Radiation therapy is generally delivered as a series of 20-30 small radiation doses, given over several weeks.

An initial irradiation selectively depletes the radiosensitive (aerobic) cell population, leaving behind a surviving population enriched in radioresistant (hypoxic) cells. As the time after irradiation increases, these hypoxic cells may reacquire the radiosensitivity characteristics of aerobic cells. This "reoxygenation" may occur through several mechanisms (Kallman, 1972). The natural variations in perfusion which lead to the development of transient hypoxia will eventually reoxygenate cells which were transiently hypoxic at the time of irradiation (Chaplin et al., 1987). In addition, radiation-induced changes in tumor blood flow, perfusion, and extravasation may improve O_2 delivery to tumor cells (Hilmas and Gillette, 1975; Reinhold, 1971). At longer times after treatment, reduced O_2 consumption by irradiated cells could allow O_2 to diffuse further through tissue. Viable malignant cells might also migrate towards areas with more favorable environments. As radiation-sterlized tumor cells die, tumor shrinkage may lead to improved perfusion of the residual tumor and to improved O_2 delivery to the remaining tumor cells. The relative contribution of these various processes may vary with the tumor and with the protocol of irradiation.

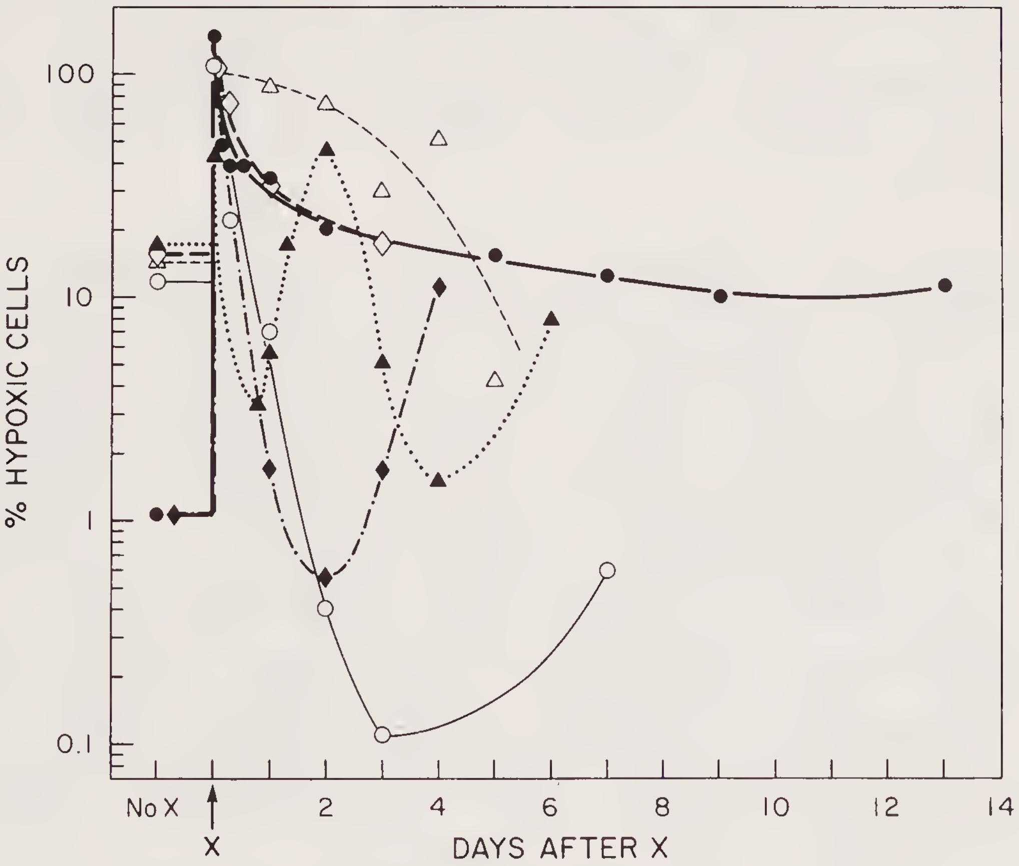

Figure 1: Patterns of reoxygenation in 6 transplanted rodent tumors given large single doses of x-rays. Reprinted from Moulder et al., 1988.

Reoxygenation patterns have been examined in several tumor systems after large single doses of radiation. In some systems, reoxygenation begins within 1 hr after irradiation and the hypoxic fraction rapidly returns to a value similar to that of untreated tumors. In other systems, reoxygenation is rapid, but the hypoxic fraction never returns to that in untreated tumors, even weeks after treatment. In one corded mammary carcinoma, the pattern of reoxygenation follows the pattern of tumor shrinkage, and the treated tumor becomes much better oxygenated than the initial tumor. In some cases reoxygenation does not begin for hours or days after irradiation. Some tumors exhibit complex changes in oxygenation, suggesting that several different processes contribute to reoxygenation.

The pattern of reoxygenation varies with the radiation dose and fractionation pattern, as well as with the tumor line (Kallman, 1972; Kallman and Dorie, 1986). One might expect more rapid reoxygenation after large doses of radiation; however, in some systems reoxygenation (Kallman, and Dorie, 1986) and microvascular improvements (Hilmas and Gillette, 1975) occur more slowly after larger doses. Reoxygenation and improved microcirculation have been observed during fractionated radiation regimens; the timing and magnitude depend upon the fractionation regimen (Kallman and Dorie, 1986; van Putten and Kallman, 1968; Reinhold, 1971).

Reoxygenation prevents solid tumors from becoming progressively more radioresistant during radiotherapy. However, this process may not result in complete oxygenation of the tumor cells during radiotherapy and need not eliminate hypoxia as a factor influencing the outcome of radiotherapy. Hypoxic cells can limit the response of experimental rodent tumors to fractionated radiation regimens (Fowler, 1983) and continuous low dose rate irradiations mimicing those used in brachytherapy (Fu, et al., 1980). This has lead to clinical trials of regimens combining radiation with agents that improve tumor oxygenation (e.g. HBO, Fowler, 1983), selectively sensitize hypoxic cells to radiation (e.g. misonidazole; Overgaard, 1988), or selectively kill hypoxic cells (e.g. mitomycin C, Weissberg et al., 1989). These trials have significant limitations, which reflect the inherent limitations of the lead compounds available for study and the compromises necessary during the design of the trials. However, several things are clear from an overview of these data. First, there is considerable heterogeneity in the patient populations: without careful stratification for site, stage, histology, and relevant prognostic factors, this heterogeneity would preclude demonstration of any effect of hypoxia. Second, for certain malignancies, multiple trials and multiple lines of evidence suggest that hypoxia limits the success of therapy and that local control rates can be improved by agents which circumvent the effects of hypoxia. Third, certain prognostic features, e.g. hemoglobin levels, may be useful in identifying patients who are at greater risk of failure and who may benefit most from hypoxia-directed interventions. Fourth, hypoxia-directed regimens improve local control in some sets of patients receiving planned post-operative radiotherapy, either to areas of known residual disease or to areas without clinical evidence of disease, but with a known statistical risk of involvement. This is in agreement with laboratory data, described above, demonstrating the presence of hypoxic cells in microscopic rodent tumors.

Reoxygenation has not been extensively studied after treatment with other modalities. However, reoxygenation would be expected after treatment with drugs such as nitrogen mustard or adriamycin, which selectively spare hypoxic cells (Hill and Bush, 1977; Tannock, 1982). In tumors treated with adriamycin, reoxygenation does occur and influences the sensitivity of the tumors to subsequent radiation treatments (Tannock, 1982). Post-operative hypoxia from compromised blood flow near surgical margins of incomplete resections could also influence the response of residual tumor to radiation and chemotherapy. Studies with a rat rhabdomyosarcoma showed that debulking this tumor did not decrease the radiation TCD_{50}, apparently because the increase in tumor hypoxia more than offset the decrease in cell number (Fischer et al., 1983). Changes in tumor oxygenation after chemotherapy and surgery should therefore be considered as possible factors influencing the results of combined modality regimens.

Oxygenation of Late Recurrences

Treatment of a tumor with large doses of radiation results in delayed degenerative damage to the stromal elements that form the tumor bed. Some tumor lines grow more slowly and develop larger proportions of necrosis when implanted into heavily-irradiated sites than when implanted in untreated sites, apparently reflecting a decreased ability of the vasculature to proliferate. In some tumors, the equilibrium between vascular volume and viable tumor volume appears similar in irradiated and unirradiated sites (Jirtle and Clifton, 1973), suggesting that the proportion of viable hypoxic cells would be similar in irradiated and control sites. However, three transplanted mouse tumor lines and a human tumor cell line xenografted into nude mice have recently been shown to have larger hypoxic fractions when implanted into previously-irradiated sites than when implanted into untreated sites (Penhanligon et al., 1987; Leith, 1988; Milas et al., 1989).

There is no good laboratory data base defining the oxygenation of experimental tumors recurring long after near-curative radiotherapy. There are data which suggest that such recurrences, and transplanted tumor lines derived from them, are more sensitive to radiation than are the primary tumors (Suit, 1966; Ando et al., 1988). This apparently reflects heritable radiation damage in the clonogenic cells, which decreases the growth rate and also increases the intrinsic cellular radiosensitivity; these changes are analogous to the heritable damage demonstrated by Sinclair (1964) in heavily-irradiated cultures. Transplanted tumor lines derived from late recurrences have hypoxic fractions similar to the parental tumor lines (Ando et al., 1988) suggesting that the intrinsic ability of the malignant cells to induce neovascularization and to survive in less-than-optimum environments are unchanged. As described above, radiation injury to the tumor bed may compromise the oxygenation of recurrent tumors in heavily irradiated sites.

OTHER MICROENVIRONMENTAL HETEROGENEITY IN SOLID TUMORS

Heterogeneity in pH

The same perfusion deficits which produce variations in oxygenation within solid tumors lead to variations in extracellular pH. This occurs in part because acidic metabolic products are not removed effectively from unperfused regions and therefore accumulate to produce acidosis (Kallinowski et al., 1988; Kennedy, 1987; Wike-Hooley et al., 1984). In addition, hypoxia per se contributes to the low pH, because of the excess lactate production and increased ATP hydrolysis occurring during hypoxic metabolism. Measurements of pH have been made in a variety of rodent and human tumors (Wike-Hooley et al., 1984). The overall range of pH measured for solid tumors is wide: 5.80 - 7.52 in rodent tumors and 5.85 - 7.68 in human tumors. Significant differences in pH have been reported between different tumors of the same type and between different types of tumors. Multiple measurements on a single tumor generally reveal significant intratumor heterogeneity. Temporal variations in pH are observed at some locations in some solid tumors (Kennedy and Rockwell, unpublished).

The ability of cells to survive and proliferate in acidic environments may be a major factor determining the ability of malignant cells to form solid tumors. Rotin et al. (1989) isolated mutant cells deficient in the Na^+/H^+ exchanger from a human carcinoma cell line and demonstrated that these mutants had lost the ability to form solid tumors in nude mice, as well as the ability to form spheroids in vitro. Drugs that interfere with the regulation of intracellular pH may prove useful in the treatment of solid tumors (Rotin et al., 1987).

Because of the activity of the Na^+/H^+ transporter and an Na^+/H^+ coupled HCO_3^-/Cl^- exchanger, the internal pH of mammalian cells generally varies less dramatically than the extracellular pH. Aerobic tumor cells in vitro maintain an intracellular/extracellular pH gradient of ~0.5 pH units when challenged by an acid environment. However, when cells are made hypoxic, the pH gradient collapses, and the intracellular pH rapidly approaches the pH of the extracellular milieu (Gillies et al., 1982). Hypoxic cells may therefore have unusually low intracellular pH as well as acidic environments.

Environmental and intracellular pH can have marked effects on the survival of cells treated with anticancer agents, which are well reviewed elsewhere (Wike-Hooley et al., 1984; Kennedy, 1987). Cells in acidic milieu have been reported to be more radioresistant than cells in physiologic environments, although this may result from proliferative changes. Low pH increases the toxicity of hyperthermia, especially at moderate temperatures, and inhibits the development of thermotolerance. The extracellular pH influences active transport and passive diffusion of drugs into cells, and thereby influences intracellular drug concentrations. The pH will alter the chemical form, and therefore the reactivity, of certain drugs: low pH decreases the cytotoxicity of adriamycin, bleomycin, and several alkylating agents, but increases the cytotoxicity of mitomycin C and certain nitroimidazoles. Heterogeneity in extracellular and intracellular pH within solid tumors would therefore be expected to contribute heterogeneity to the response of the tumor cells to treatment.

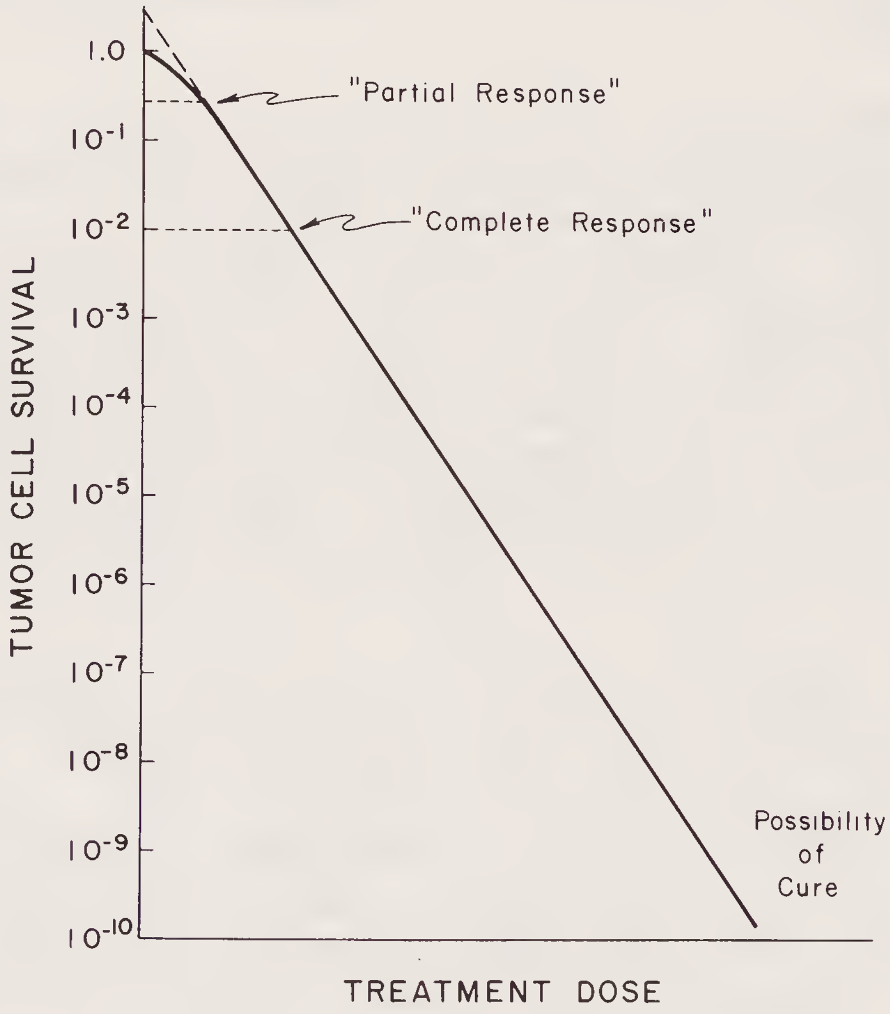

Figure 2: Even small tumors contain large numbers of cells. All the clonogenic tumor cells must be killed to cure the tumor. This means that the surviving fraction at the end of therapy must be reduced to values below ~ 10^{-9}. Even very small subpopulations which are resistant to therapy will limit tumor cure, even though they may not limit the attainment of partial and even complete responses.

Heterogeneity in Drug Delivery

Heterogeneous distributions of active drugs within solid tumors also contribute to variations in cell survival. Studies of the intratumor distribution of intraveneously-injected dyes (Goldacre and Sylven, 1962; Chaplin et al., 1985) show that large molecules, even stable ones, do not distribute uniformly within solid tumors, and that some viable, as well as necrotic, regions remain unstained. The distribution of active forms of short-lived drugs might well be even less uniform. The implications of inhomogeneous drug distributions are currently receiving intensive investigation in a number of laboratories.

THERAPEUTIC IMPLICATIONS OF ENVIRONMENTAL HETEROGENEITY

Environmental factors are critical in determining the sensitivity of cells to radiation, chemotherapy, and other antineoplastic agents. Microenvironmental heterogeneity within solid tumors will therefore be an important factor limiting the response of these tumors to treatment. Microenvironmental changes occurring during the course of multi-treatment regimens will influence the sensitivity of residual tumors to the later treatments. Although the major genetic and environmental subpopulations will dominate the response of tumors when partial responses, and perhaps even complete responses, are analyzed, even very small resistant subpopulations can limit tumor control. Microenvironmental factors must therefore be considered in planning treatment regimens intended to cure solid tumors.

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THE NATURE OF TUMOUR HYPOXIA: IMPLICATIONS FOR THERAPY

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INTRODUCTION

As malignant cells divide within the body to form a solid tumour mass, their viability and susceptibility to therapeutic attack become dependent on the developing vascular system within the cellular aggregate. For radiation therapy the homogeneous delivery of oxygen is of critical importance since hypoxic cells are known to be three times more resistant to x-rays than oxic ones. For chemotherapeutic attack, delivery of the systemically administered agent to all the cells within the tumour is one of the important parameters which will influence the therapeutic outcome. The question we have attempted to address over the last few years is how do poorly perfused microregions arise in solid tumours?

For simplicity, we have assumed that inadequately perfused areas are synonymous with regions of low oxygenation which are radiobiologically hypoxic. Hypoxic cells and, by analogy, poorly perfused tumour microregions can occur in two different ways (Fig. 1). The classical model is that such cells exist distant from the functional vasculature and, in this case, oxygen depletion by intervening cell layers results in hypoxia. However, transient stoppages in blood and thus erythrocyte flow will also produce hypoxia. Until recently, direct determination of the nature of hypoxia within a three dimensional tumour mass was not possible. However, recent developments in flow and static fluorescence cytometry pioneered in our laboratories have indicated the existence

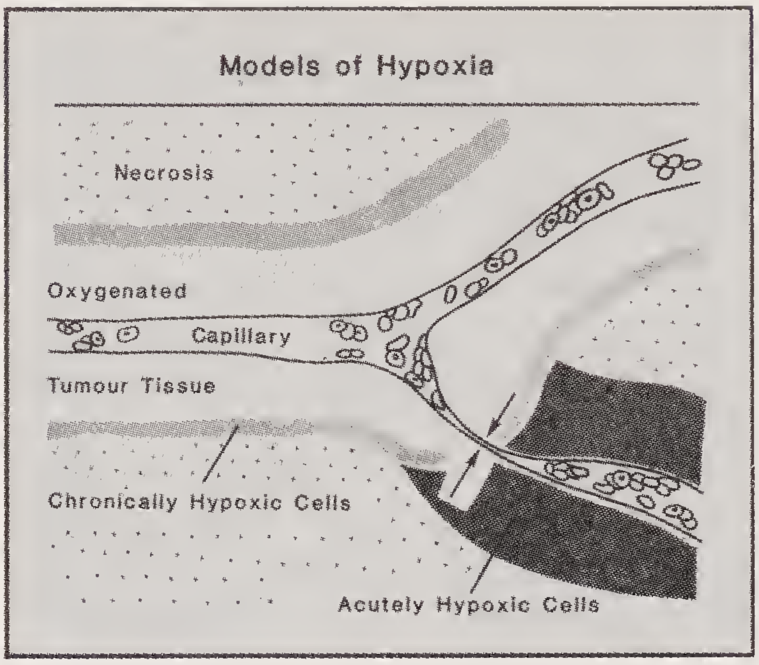

Figure 1:

Schematic representation of the two possible ways hypoxic regions can arise within a tumour. Chronically hypoxic cells exist in a rim distant from the blood vessel, while acutely hypoxic cells arise because of the temporary closure of a tumour blood vessel.

of hypoxia resulting from transient fluctuations in microregional perfusion (Chaplin et al. 1985, 1986, 1987, 1989c; Trotter et al. 1989). This finding has implications for the method of choice to reduce or eliminate the impact of hypoxic cells. For instance, such 'acute' hypoxia would be refractory to 'reoxygenation' using techniques designed to increase the oxygen carrying capacity of blood, e.g. hyperbaric oxygen breathing or use of perfluorochemical emulsions. Also, such regions, if not perfused for long periods, would provide sanctuaries for cells against certain chemotherapeutic agents whose tissue penetration may be limited. Thus, approaches designed to reduce or eliminate this microregional heterogeneity in tumour blood flow could have roles as adjuvants for both radiation therapy and chemotherapy. In contrast to eliminating tumour hypoxia, there has been increasing interest in inducing homogeneous hypoxia in tumours (Chaplin, 1986, 1987, 1989a). The strategy behind this approach is to administer a bioreductive drug (i.e. an agent which is reduced under anoxic conditions to a toxic metabolite) and then induce hypoxia selectively in the tumour. Tumour hypoxia can be dramatically increased by administration of a variety of vascactive agents including 5-hydroxytryptamine and hydralazine. A brief review of the ongoing work being undertaken in our lab on the occurrence of tumour hypoxia, its elimination, and its exploitation is given here.

MATERIALS AND METHODS

(i) Mice and tumour

Studies used the SCCVII squamous cell carcinoma or KHT sarcoma implanted in C_3H mice and Lewis lung carcinoma implanted in C57BL mice. Tumours were implanted subcutaneously over the sacral region of the back and used when they attained a size of 400-600 mg.

(ii) Irradiation procedures

Irradiation was carried out without anaesthesia in a manner similar to that described previously (Chaplin et al. 1983) using either 270 kVp x-rays at a dose rate of 1.5 Gy min^{-1} or 250 kVp x-rays at a dose of 2.8 Gy min^{-1}.

(iii) Fluorescent stains

Hoechst 33342 (purchased from Sigma Chemical Co., St. Louis, Mo.) was dissolved in sterile phosphate buffered saline (PBS) and injected IV at a dose of 10-20 μg/g. The carbocyanine dye $DiOC_7(3)$ (purchased from Molecular Probes Inc., Eugene, Oregon, U.S.A.) was dissolved in DMSO, diluted to 75% DMSO with phosphate buffered saline and injected at a dose of 1 μg/g.

(iv) Flow cytometry studies

For flow cytometry studies, Hoechst 33342 was administered intravenously to tumour bearing animals in one of two protocols, (a) as a bolus injection 20 minutes prior to radiation, or (b) as an infusion during radiation treatment. Twenty minutes after radiation treatment, the tumours were excised, enzymatically disaggregated into a single cell suspension and then sorted into subpopulations on the basis of Hoechst fluorescence using a

Becton-Dickinson FACS 440. After sorting, cell survival was assessed using the soft agar clonogenic assay. Full details of this procedure have been described previously (Chaplin et al. 1987)

(v) Fluorescence imaging studies

For these studies, Hoechst 33342 was injected IV into tumour bearing mice. This was followed 20 minutes later by an injection of $DiOC_7(3)$. Animals were sacrificed 5 minutes after the last injection and the tumours frozen at -20°C. Frozen sections 10 μm in thickness were then examined using a Zeiss microscope with an epicondenser, neofluor objectives and a 100 W mercury light source. This microscope system is linked to an IBM-AT computer-based image processing system which enables images to be stored and analyzed. Full details of this procedure are described elsewhere (Trotter et al. 1989).

(vi) Drugs

Nicotinamide (purchased from Sigma) was dissolved in sterile PBS and injected IP at a dose of 1 mg/g. Hydralazine was supplied by Ciba-Geigy (Mississauga, Canada) dissolved in sterile PBS and injected IP at a dose of 5 μg/kg. RSU 1069 was supplied by Warner Lambert (Ann Arbour, U.S.A.) dissolved in sterile PBS and injected IP.

RESULTS

(i) The nature of tumour hypoxia

Evidence for intermittent hypoxia occurring in experimental tumours has been obtained using recently developed flow and static fluorescence imaging techniques. These studies utilize the DNA binding/diffusion properties of the bisbenzamide stain Hoechst 33342. This property results in cells close to the functional blood supply, after intravenous injection into tumour bearing animals, being very brightly stained while cells distant from the functional vasculature being dimly stained (Chaplin et al. 1985). In addition to the binding pattern, the short plasma half life (110 secs), and the good cellular retention during disaggregation procedures make this compound ideal to isolate cells close to the functional vasculature at particular instances in time (Olive et al. 1985). When the Hoechst is infused during irradiation, the cells most intensely stained should display an oxic response, whereas the more dimly stained cells should show a more radioresistant response. However, when the Hoechst is injected 20 minutes prior to irradiation some vessels which were perfused immediately after injection may close down or become occluded prior to radiation. Thus, cells which are intensely stained will show a relatively radioresistant response since some will be hypoxic during irradiation. Typical results for 400-600 mg SCCVII and KHT tumours after 10 Gy of x-rays are shown in Figure 2. Large differentials are seen between the radiation response of the brightest 10% of cells and the dimmest 10% (based on Hoechst fluorescence) for the infusion protocol, with the brightest cells being more radiation sensitive. However, for the injection protocol, the difference is lost or reduced. These results provide evidence for hypoxia resulting from blood flow changes within tumours.

(ii) Fluorescence imaging studies

Although the flow cytometry data described above clearly indicate vessels closing, they cannot easily provide data on vessels opening. Direct evidence for both vessel opening and closing can be obtained using histologic methods. This technique which is described in detail elsewhere utilizes the diffusion, binding and distribution half-life properties of two fluorescent stains (Hoechst 33342 and $DiOC_7(3)$) with different excitation/emission properties. Separation of the administration of the two stains by a period of 20 minutes facilitates visualization of the changes in functional blood flow patterns. Figure 3 shows a digitized image of a tumour section in which the blood flow has changed between injections. Using this approach, the vessel mismatch (i.e., number of vessels containing one stain and not the other) can be quantified. Results for SCCVII tumours 500 mg in size indicate that 8.9% of vessels are mismatched if the stains are injected 20 minutes apart.

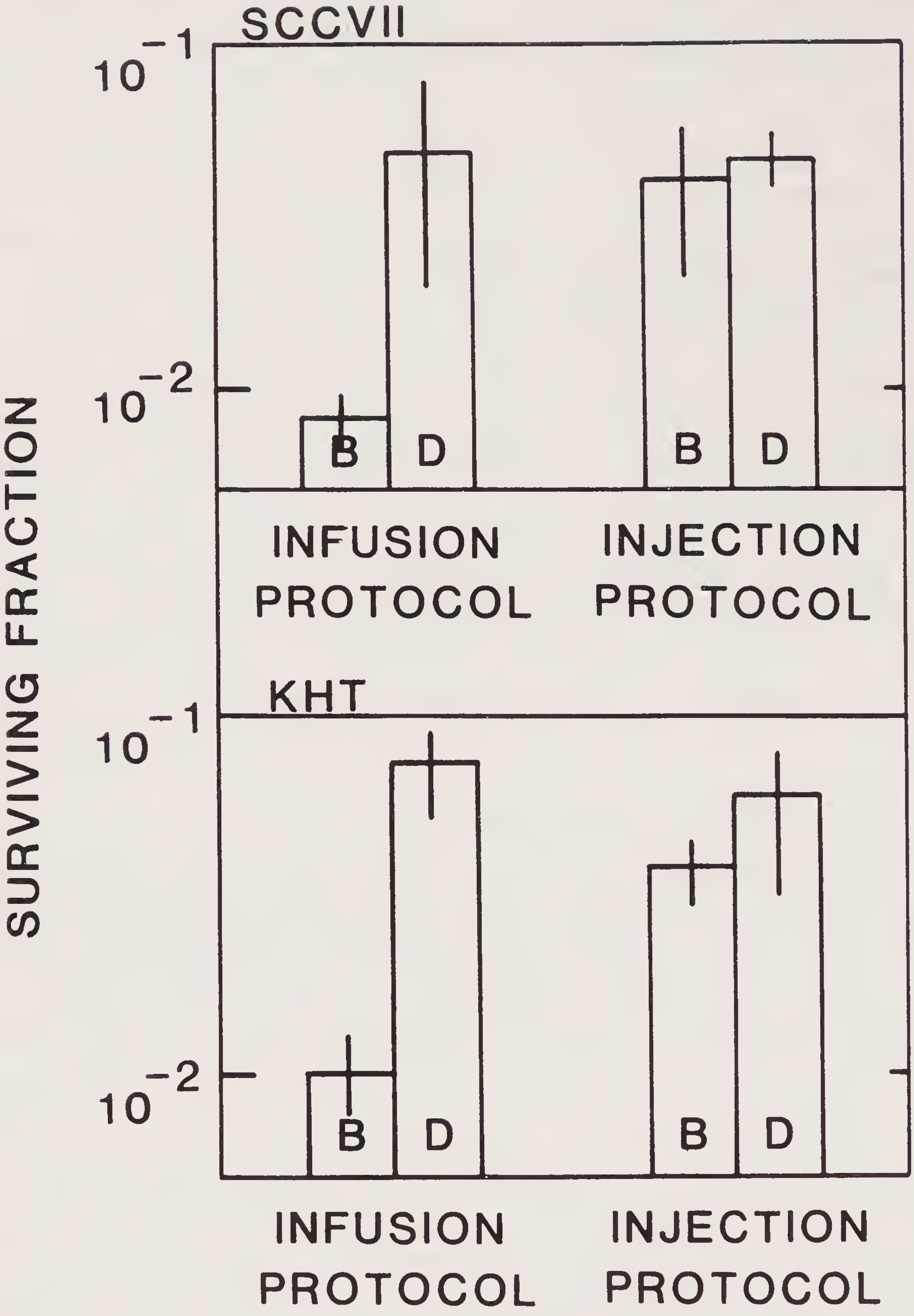

Figure 2

The survival of SCCVII and KHT tumour cells in the brightest and dimmest sort fractions after 10 Gy of x-rays. Large differential in survival was seen between brightly (B) and dimly (D) staining cells for the infusion protocol. However, for the injection protocol, little or no differential in survival was observed.

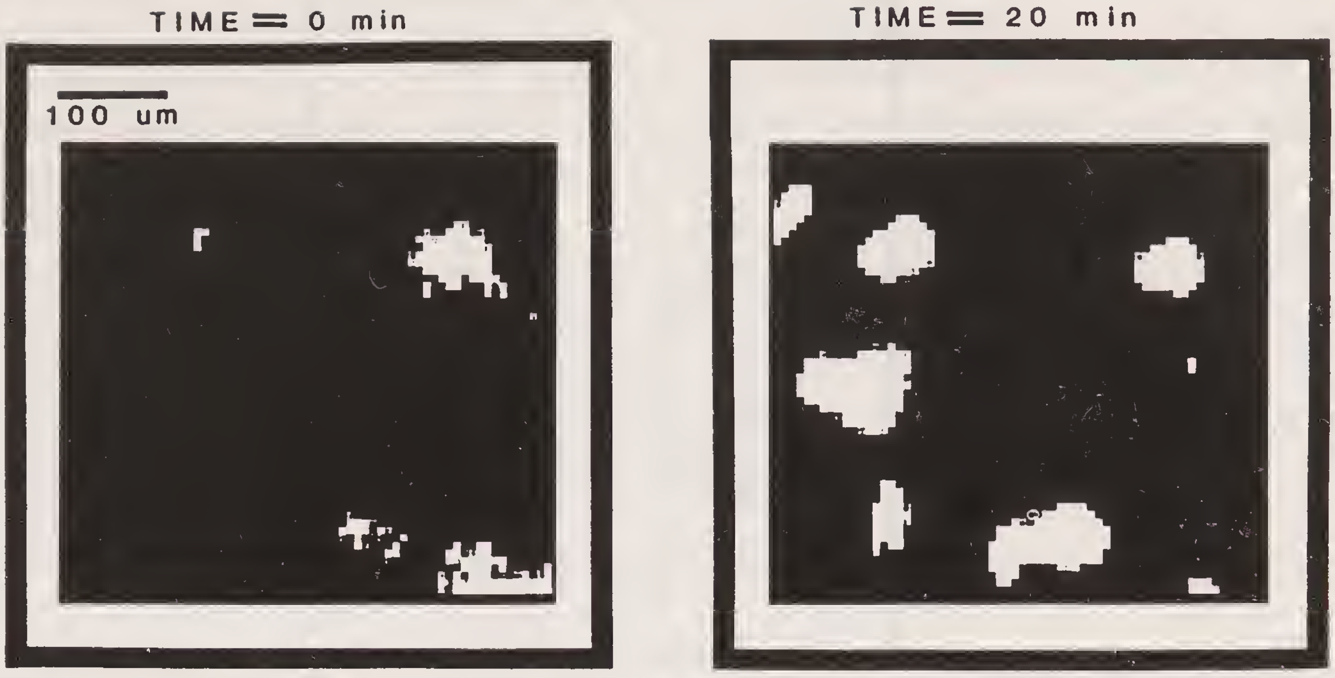

Figure 3

Evidence for vessel opening in the SCCVII tumour. The left and right panels depict Hoechst 33342 and $DiOC_7(3)$ stained vasculature in a given tumour microregion. In this experiment $DiOC_7(3)$ was administered 20 minutes after Hoechst 33342. Evidence for vessel closing can also be obtained using this technique.

(iii) Approaches to reducing microregional heterogeneity in tumour blood flow

We are currently evaluating several agents for their ability to reduce acute hypoxia resulting from transient fluctuation in blood flow within the tumour. One of the most effective agents so far identified is nicotinamide. We chose to evaluate this agent because of its reported ability to reduce tumour hypoxia and increase the radiation response of a variety of experimental tumours (Horsman et al. 1987, 1989). The effect of administering nicotinamide at a dose of 1.0 mg/g on transient perfusion as assessed by our cell sorting and histology techniques is clearly shown in Fig. 4 and Table 1. In Fig. 4 the nicotinamide was administered at time 0, 40 minutes later the Hoechst was administered followed in 20 minutes by x-rays. The results show that the survival of the brightest fraction of cells is reduced to a level similar to that seen for the infusion protocol in Fig. 2

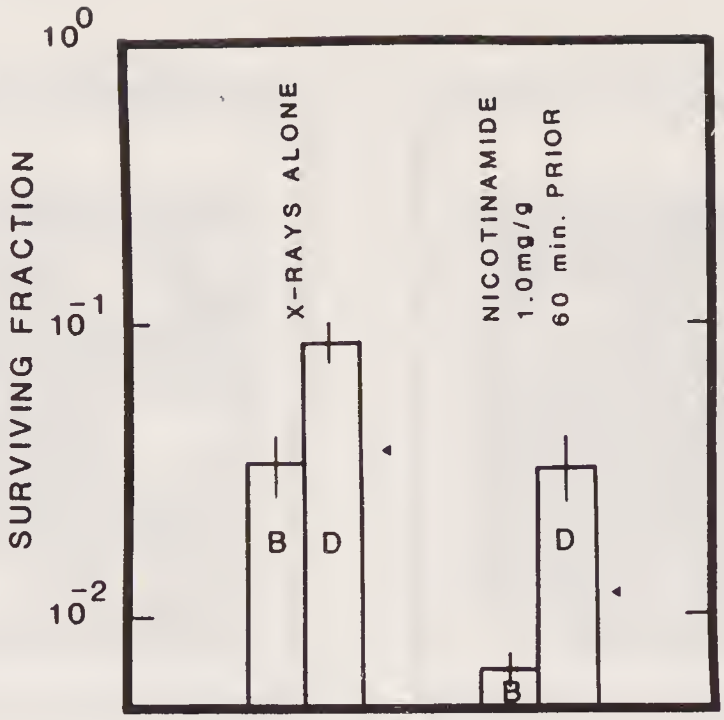

Figure 4

The survival of SCCVII tumour cells in the brightest and dimmest sort fraction after 10 Gy of x-rays alone or 10 Gy of x-rays given 1 hour after nicotinamide 1.0 mg/g IP. It can be seen that nicotinamide sensitizes the brightly staining cells (B) in the injection protocol.

Table 1

The effect of nicotinamide 1.0 mg/g on perfusion mismatch in SCCVII tumours. Errors represent standard errors of the mean values.

Control (T=20) (n=10)	10.3 ± 1.3 %
Nicotinamide 60 min. prior (n=5)	2.0 ± 0.32 %
Simultaneous stain adm. (n = 11)	1.3 ± 0.15 %

indicating that the number of vessels closing between Hoechst injection and irradiation is reduced by prior administration of nicotinamide. This fact is confirmed in the histological studies summarized in Table 1 where nicotinamide was administered 60 minutes before Hoechst and then 20 minutes later the carbocyanine stain $DiOC_7(3)$ was given. The percentage of vessels mismatched, i.e. having one stain and not the other is dramatically reduced compared to saline controls (i.e. 2.0 vs. 10.3). Although the mechanism for this effect observed with nicotinamide is as yet unknown, the phenomenon observed could have important implications for radiation and drug treatment of tumours. Further studies are now planned to evaluate the efficacy in radiation treatment regimes of combining a strategy which eliminates acutely hypoxic cells (e.g. nicotinamide) with a strategy which should increase the oxygen carrying capacity of the blood and thereby reducing chronic hypoxia (e.g. perfluorochemical emulsions).

(iv) Therapeutic potential of increasing tumour hypoxia

As described above, there is a clear rationale for developing strategies to reduce or eliminate tumour hypoxia. However, there is now evidence that in certain situations, increasing tumour hypoxia may be of therapeutic benefit. The development of drugs which are selectively toxic to hypoxic cells and the finding that certain vasoactive agents can selectively reduce tumour oxygenation, have led to the design and testing of novel therapeutic schedules. The initial work involved administration of the hypoxic cell cytotoxin to the animal and then selectively creating additional hypoxia within the tumour using such agents as 5-hydroxytryptamine or hydralazine (Chaplin 1986, 1987, 1989a). Typical results obtained, combining the hypoxic cell cytotoxin RSU 1069 with hydralazine, are shown in Fig. 5. In this figure, the fraction of tumour cells surviving 18 hours after treatment is shown. Hydralazine, which has no effect on cell survival when given alone, increases the effectiveness of RSU 1069 by a factor of 3-4. This increased efficacy against the tumour occurs without increased systemic toxicity indicating therapeutic gain (Chaplin and Acker, 1987; Chaplin, 1989). Additional studies indicate that appropriate timing of the induction of tumour hypoxia by hydralazine can increase the effectiveness of RSU 1069 when used in combination with radiation or hyperthermia (Chaplin 1988; Horsman et al. 1988). Another consequence of induction of tumour hypoxia is a decrease in environmental pH as a result of increased anaerobic glycolysis. This decrease could provide an additional selective target for cancer chemotherapy because some agents such as chlorambucil are known to be more toxic at reduced pH (Mikkelson 1982). Furthermore, there is in vitro evidence that the combination of an acidic pH and hypoxia can reduce cell viability in the absence of treatment, and also increase the effectiveness of the alkylating agent melphalan (Rotin et al. 1986; Chaplin et al. 1989b). Thus, there is a need to investigate the potential of inducing tumour hypoxia to potentiate existing treatment regimes in vivo.

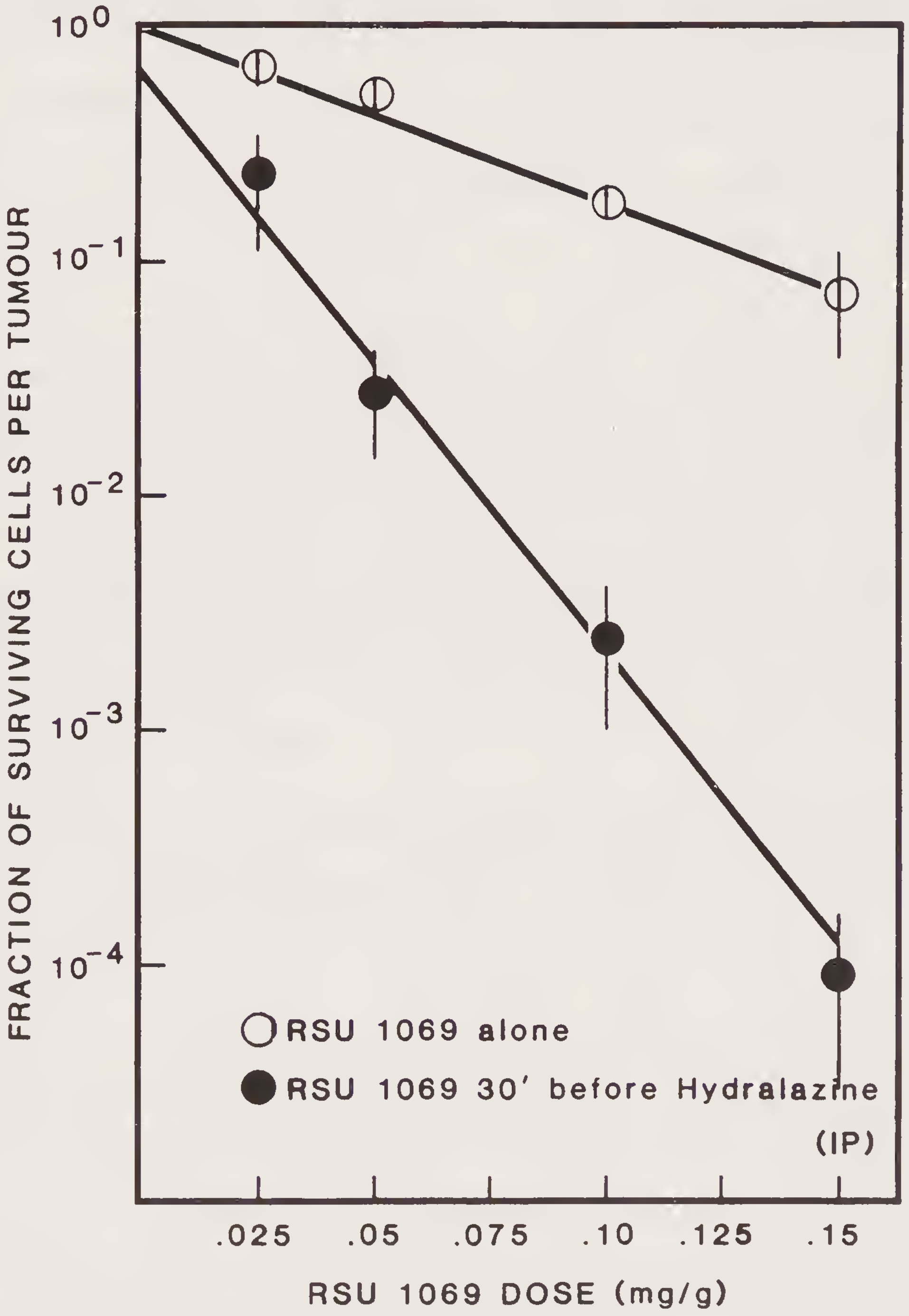

Figure 5

The effect of hydralazine (5 mg/kg) administered immediately after various doses of RSU 1069 on tumour cell survival in the Lewis lung carcinoma.

DISCUSSION

There is clear evidence from previous work that the inadequacy of the vascular system leads to areas which are poorly perfused and, as a result, are hypoxic. The present studies provide further insights into the physiology of the tumour and how this can impact on therapeutic outcome. New approaches to improving tumour perfusion and oxygenation may eventually lead to improved responses to existing chemotherapy and radiotherapy regimes. Paradoxically, it may be that the inadequacy of the tumour vascular system can provide a key to designing novel therapeutic strategies.

ACKNOWLEDGEMENTS

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INTEGRATING THE CONCEPT OF DOSE INTENSITY INTO A STRATEGY FOR SYSTEMIC THERAPY OF MALIGNANT DISEASE

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INTRODUCTION

In 1969, major impetus was given to solid tumour chemotherapy when a 90% response rate was reported in women with advanced breast cancer treated with a 5-drug regimen (1). Others have since confirmed that combination chemotherapy can cause disease regression and palliate symptoms of advanced breast cancer. In addition, after surgical removal of tumor in women with apparently localized breast cancer, chemotherapy delays recurrence and prolongs survival (adjuvant chemotherapy). However, in the past decade, results of chemotherapy of breast cancer have not improved further and combinations of different drugs and schedules have failed to break the impasse. Unfortunately, introduction of these combinations and schedules has obscured important dose-response relationships.

We have analyzed the literature and have suggested one approach to rediscovering dose-response relationships and which might improve treatment results (2). By use of simple arithmetic, one can precisely characterize the "strength" of chemotherapy regimens which employ agents singly or in combination. To describe this "strength" we have adopted the term "dose intensity". Dose intensity correlates with outcome in breast cancer and other diseases (3).

For regimens containing only one drug, dose intensity can be calculated simply by translating the drug dosage specified in the protocol into a standard form:

$mg/M^2/wk$, regardless of the schedule used*.

For regimens containing more than one drug, dose intensity can also be calculated. For example, consider a series of 3-drug regimens each of which contains cyclophosphamide (C), methotrexate (M) and 5-fluorouracil (F). First, we arbitrarily designate any member of the series of CMF regimens as the "standard" and, for each of the other regimens, express the dose intensities of C, M and F as decimal fractions of the dose intensities of C, M and F in the "standard".

These decimal fractions are then averaged for each CMF regimen to give the "average relative dose intensity" for that regimen (see table).

Average relative dose intensity of CMF regimens calculated in this way correlates with outcome of combination chemotherapy in advanced breast cancer and early breast cancer (3). Regimens missing one or two of the drugs in the standard regimen can still be incorporated into the series by assigning a dose intensity of zero to the missing drug(s). A similar approach to analysis of combinations of other drugs besides CMF in breast cancer and of various drug combinations in other malignant diseases also demonstrates a relationship between average relative dose intensity and outcome (3). In addition, the concept of dose intensity can be used to determine the degree of activity of any particular drug in a combination and thus may lead to design of more effective regimens (3).

In order to calculate dose intensity, it is necessary to completely ignore scheduling i.e. to assume scheduling does not directly determine anti-tumour effect. Most likely, scheduling is important because it determines toxicity and, thereby limits the dose intensity which can be reached. Hence, scheduling determines anti-tumour

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*"Scheduling" refers to the disposition of drug doses over the time of treatment ie: whether daily, weekly, monthly, etc. However, data from studies using infusions should not be mingled with results from pulsatile methods of administration.

TABLE **1**

Sample Calculation

TEST REGIMEN	Step 1 Convert to standard form $mg/M^2/wk$	Step 2 Express as fractions of corresponding dose intensities in standard regimen*	Step 3 Calculate fractions and average them
Cyclophosphamide 100 $mg/M^2/d$ Day 1-14 q28d	350	350 / 560	.62
Methotrexate 40 mg/M^2 Day 1 & 8 q28d	20	20 / 28	.71
Fluorouracil 600 mg/M^2	300	300 / 480	.62
		$\bar{x}$ =	.65

* - assume standard regimen to be CMF content of CMFVP regimen of Cooper et al (1).

effect, but only indirectly. This is probably a correct assumption for most drugs except cytosine arabinoside and perhaps also methotrexate. In addition, it remains to be determined whether, and to what extent the total dose of drug administered is a determinant of outcome independent of dose intensity.

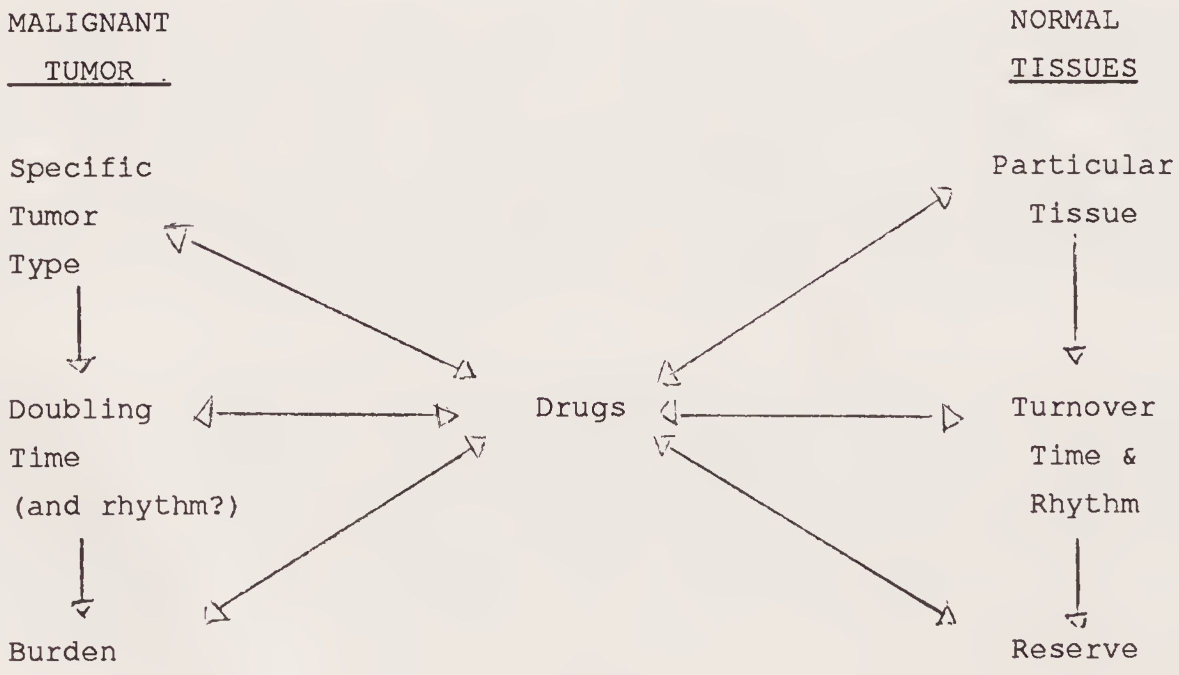
The concept of dose intensity is widely applicable and should be taken into account in designing or analyzing chemotherapy regimens. We would predict that maneuvers to increase dose intensity will improve treatment results. However, randomized clinical trials are required to delineate where these maneuvers are appropriate and where not (4). In addition, it is important to relate the concept of dose intensity to the other concepts regarding systemic therapy of malignant disease.

DOSE INTENSITY AND THE CELL KILL HYPOTHESIS

According to the cell kill hypothesis, systemic therapy of cancer will be successful only to the degree it produces a net reduction in tumor cell burden. To achieve this, a plethora of systemic cytotoxic treatments is currently available. Figure 1 suggests there are two parallel heirarchies of factors which determine outcome of these systemic treatments.

Figure 1

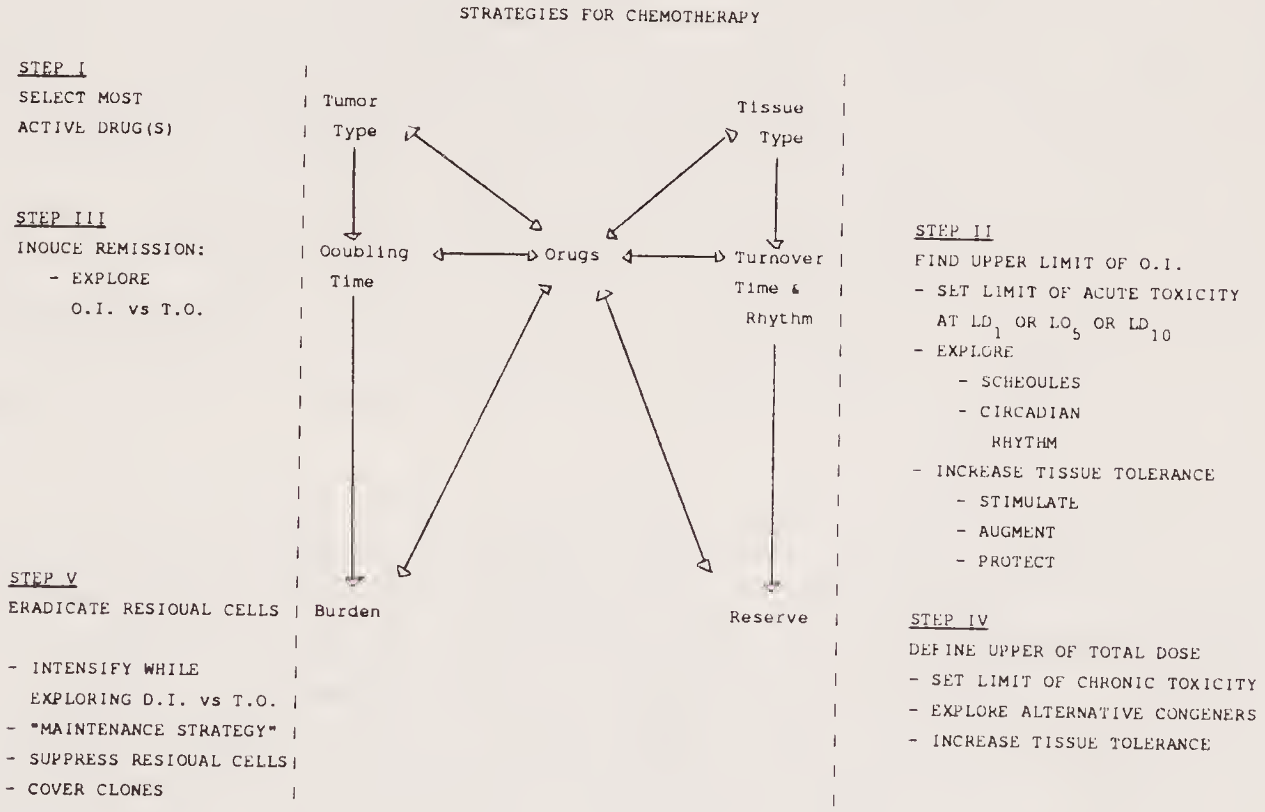
FACTORS DETERMINING OUTCOME OF CHEMOTHERAPY



According to the figure, treatment outcome is primarily dependent on the sensitivity of individual tumor and normal cells to the cytotoxic drugs(s), secondarily dependent upon the growth rate and rhythm of tumor and normal tissues, and finally dependent upon the size of tumor burden and normal tissue reserves.

This paradigm suggests three broad treatment strategies. First, the most active least toxic drug(s) must be selected. Second, remission must be induced by an optimal combination of dose intensity and total dose of the chosen drug(s) given to a limit of permissible toxicity specified at the outset of the investigation. Thirdly, the residual tumor burden must be eradicated without incurring an unacceptable long-term deficit in normal tissue functioning. Figure 2 superimposes these strategies upon Figure 1 and indicates how these strategies could be implemented, and in what order.

Figure 2


STRATEGIES FOR CHEMOTHERAPY
STEP I
SELECT MOST ACTIVE DRUG(S)
STEP III
INDUCE REMISSION:
- EXPLORE D.I. vs T.O.
STEP V
ERADICATE RESIDUAL CELLS
- INTENSIFY WHILE EXPLORING D.I. vs T.O.
- "MAINTENANCE STRATEGY"
- SUPPRESS RESIDUAL CELLS
- COVER CLONES
Tumor Type
Doubling Time
Burden
Drugs
Tissue Type
Turnover Time & Rhythm
Reserve
STEP II
FIND UPPER LIMIT OF D.I.
- SET LIMIT OF ACUTE TOXICITY AT LD_1 OR LD_5 OR LD_{10}
- EXPLORE
- SCHEDULES
- CIRCADIAN RHYTHM
- INCREASE TISSUE TOLERANCE
- STIMULATE
- AUGMENT
- PROTECT
STEP IV
DEFINE UPPER OF TOTAL DOSE
- SET LIMIT OF CHRONIC TOXICITY
- EXPLORE ALTERNATIVE CONGENERS
- INCREASE TISSUE TOLERANCE

STEP I

In the first instance, the most active drug or drugs should be used. To make this selection, we should rely whenever possible on clinical data from well conducted clinical trials. We should also assume that biochemical pharmacologic principles apply as much to normal as to malignant cells, with regard to drug transport rates, cellular levels of various target enzymes, drug retention, etc. Thus, synergistic combinations or sequencing of drugs can be expected to produce increases in lethal effects on **normal** cells, as well as the target tumor cells, even though such combinations or sequences may have been identified from studies of **tumor** cells in model systems. Indeed, in the absence of direct clinical data to the contrary, we might choose to deliberately avoid the most cytocidal combinations, sequences, or schedules until randomized trials identify that such combinations are indeed beneficial in the clinical setting. Viewed from this perspective, the simplest and most logical decision might be to initially choose the single most active drug and rely on that for remission induction.

STEP II

After selecting the most active drug(s), a series of investigations would be required to define the upper limit of dose intensity which can be tolerated by the host over a reasonable period of time of induction treatment e.g. 12 - 16 weeks. However, as shown in figure 2, before such limits can be defined, a major decision must be made regarding the upper level of acute toxicity which will be accepted. This could be LD_1 (or less), LD_5, or LD_{10}. Next, attention should be paid to optimal scheduling to determine if the drug(s) were best given daily, weekly, monthly etc. to produce the least toxicity for the highest dose intensity over the induction period of 12-16 weeks.

While scheduling is being explored, attention should also be paid to the timing of drug delivery in relation to the diurnal (circadian) rhythms of the normal tissues. It may be possible to take advantage of the fact that there will be periods of the day when the proliferating compartments in the normal tissues will be relatively quiescent and therefore may be **more** drug resistant. The combination of the proper schedule and optimal diurnal timing could allow clinically important increments of dose intensity to be introduced without commensurate increases of toxicity.

In addition, further increments of dose intensity could be introduced by a variety of maneuvers designed to increase normal tissue tolerance to the drugs(s), still keeping toxicity within the pre-set limit of LD_1 or $_5$ or $_{10}$. Such maneuvers could include expansion of normal reserves by stimulation with cytokines, restoration of depleted tissue reserves by transfusion of end-product blood cells, stem cells from blood or autologous marrow, and by simply increasing host tolerance for myelosuppression with oral antibiotics (e.g. one of the new quinolines) to prevent infections during periods of neutropenia.

STEP III

Once the maximum possible dose intensity has been incrementally achieved up to the pre-set limits of toxicity, a matrix of dose intensities and total doses should then be explored to identify the optimum **combination** required for remission induction.

Throughout the various phases of drug treatment, dose intensity and total dose will probably both be important and independent determinants of cytotoxic effect. In early phases of treatment (during remission induction), dose intensity might be the more important because faster growing tumor cells might predominate in the tumour while the susceptible normal cells have not yet been recruited into cycle to repair damage from chemotherapy.

STEP IV

After remission induction, but before attempting eradication of residual tumor cells, it would be important to define the limit of acceptable chronic toxicity which can be expected from further use of chemotherapy. These limits have just begun to be defined with drugs such as bleomycin, adriamycin and cisplatinum which damage the lungs, heart, and kidneys, respectively (3). Even for these drugs, the total doses permissible have not been precisely identified. For other drugs, we have only just started to appreciate the relationship between long-term side effects on the one hand and total dose, and scheduling on the other. Identification of protective agents or use of new congeners of existing cytotoxic agents are also being explored as means of reducing long-term side effects.

STEP V

After induction of remission with chemotherapy using the correct combination of dose intensity and total dose, a residual tumor burden may persist and require further systemic treatment. This residual cell mass may have a growth rate lower than the starting tumor, due to selective killing of faster growing cells during remission induction. If so, longer time gaps would be permissible between treatments. This would lower the dose intensity, but allow for a much longer duration of treatment thereby raising the total dose. Thus, drawn-out treatments eventuating in high total doses could be used such as the 2-3 years of **maintenance** chemotherapy in acute lymphoblastic leukemia of childhood. Alternatively, post-induction treatments could rely on **suppression** of residual tumor cells with hormones or other differentiating agents such as retinoids.

Regardless of growth rate considerations, it seems reasonable to assume the cells in the residual tumor would be **relatively** resistant to the dose intensity of the original induction regimen and that in addition, some of the tumor cells would be mutants and therefore resistant to the initial inducing drug(s) by an order of magnitude. It would be logical to greatly increase the dose intensity of the treatment to overcome the relatively resistant cells while also adding non-cross-resistant drugs to cover the mutant clones. The probability of resistant mutants being left behind as harbingers of relapse would be especially high in the larger tumors. Thus, one could intensively consolidate with several drugs simultaneously as with megadose-then-marrow rescue or one could give the drug combination 3 to 4 times at 1/2 megadose levels without marrow rescue. This latter approach would give an intermediate increase in dose intensity but allow a larger total dose to be given thereby avoiding the toxicity and expense of the megadose-then-marrow rescue approach while retaining the efficacy.

Another intensification strategy would be augmentation of immune attack upon a minimal residual tumor burden as by stimulation with agents as interferon or levamisole, or passive boosting of immunity with infusion of monoclonal antibodies or stimulated autologous lymphocytes.

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MODELLING THE EFFECT OF CHEMOTHERAPY INTENSITY

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INTRODUCTION

Hypotheses can play a useful role in research by providing a framework for understanding observations, and by suggesting appropriate prospective tests of validity of the chosen model (Huszagh and Infante, 1989). The number of variables required to provide a complete description of chemotherapy makes intuitive relationships unreliable. For example, both total drug dose (Bonadonna and Valagussa, 1981) and "dose intensity" (Hryniuk and Levine, 1986) have been retrospectively correlated with outcome, but it has remained unclear to what extent these parameters might be expected to relate to each other from first principles.

We have recently developed a mathematical model using common fundamental assumptions such as first-order kinetics (Coldman et al, 1988). The generalized model suggests that total dose and treatment intensity cannot be completely separated when comparing one chemotherapy protocol with another. This feature represents an important distinction from the claim that intensity alone may be sufficient (Hryniuk, 1988). For those without a mathematical background, a simplified log-linear model will be presented which permits a more visual representation at the price of more restrictive initial assumptions.

THE SIMPLIFIED MODEL

The initial assumptions of the simplified model are:

*1. Exponential tumour growth
*2. Instantaneous cell kill
*3. Regrowth equals growth rate
4. All cells are uniformly drug sensitive
5. Constant fraction kill (first-order)
*6. Linear relation of drug dose vs log kill
*7. Single drug or fixed drug ratio
(* Not essential for the generalized model)

Some of these assumptions are shown schematically in Figure 1. The linear dose-response relation (assumption 6) underlies the consolidation of dose and time understood by the dose-intensity concept which expresses treatment as $mg/m^2/week$ regardless of schedule, and treats dose reduction and treatment delay as being equivalent.

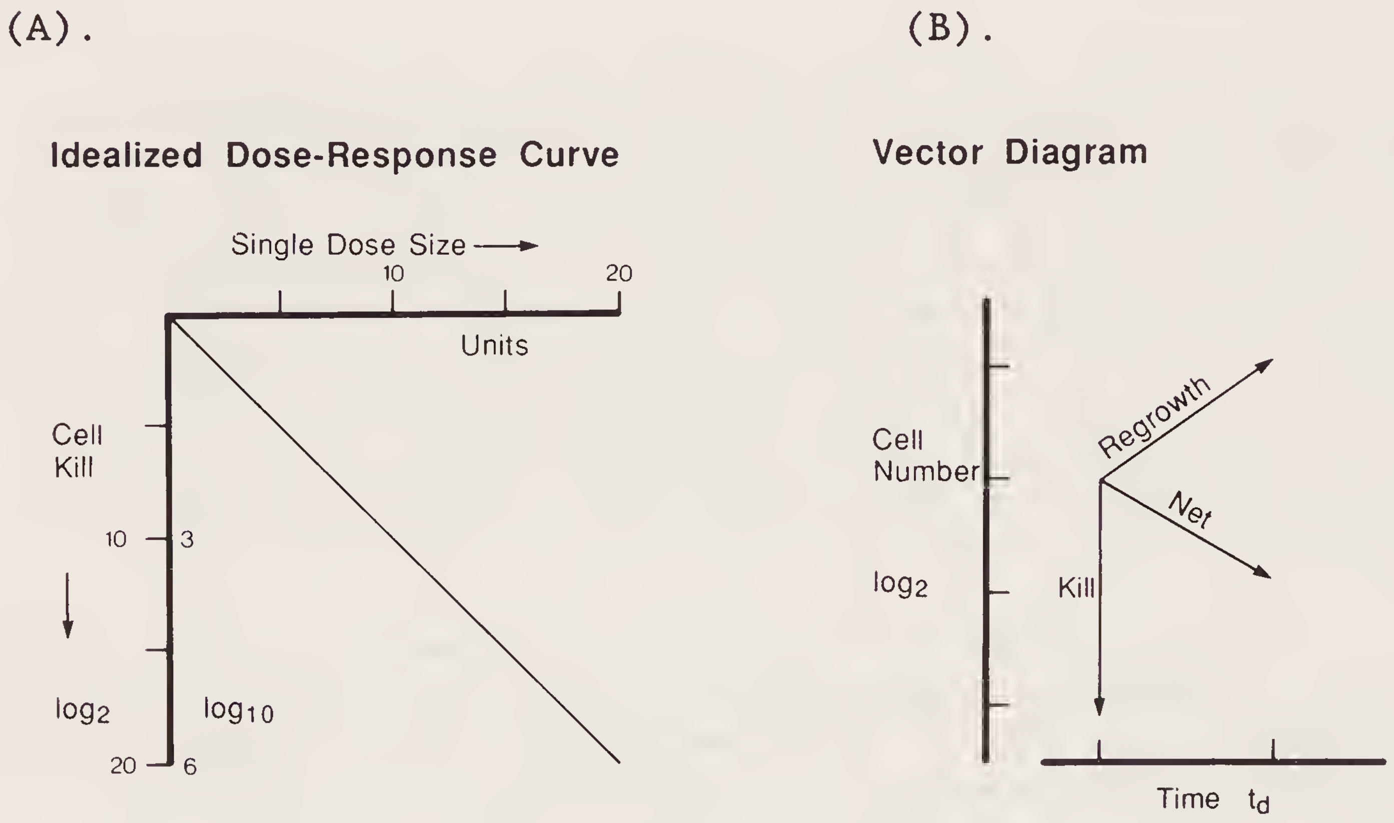

Figure 1. (A) Log cell kill is proportional to the drug dose. One unit of dose is defined as that producing a 50% cell kill (10 units thus result in 2^{10} or ~1000-fold kill). (B) Net kill is the resultant of the (assumed) instantaneous cell kill and the subsequent regrowth. The cell growth or cell kill is expressed in units of $\log_2$ (doublings or halvings respectively). The time axis is in doubling times t_d.

DEFINITIONS

Table 1 lists the symbols to be used in the following discussion. In order to derive simple quantitative relationships, all units will be in multiples or factors of two for linear or log scales respectively (Fig.1). From assumptions 1 and 3, tumour growth and regrowth are exponential with a constant doubling time, t_d. From assumption 6, log cell kill is proportional to linear increments in dose, and one unit of dose, u, is defined as that which kills 50% of sensitive cells. On this basis, dose intensity I is normalized as drug units per doubling time, u per td.

TABLE 1. Definitions and symbols.

t_d = tumour doubling time
u = drug dose, 1 u causes 50% cell kill
n = number of cycles
i = interval between doses (in doubling times)
TD = total dose = u x n
I = normalized dose intensity, u per t_d
= TD/(i x n) = u/i
P_S = population (number) of drug sensitive cells
P_R = population (number) of drug resistant cells
$P_{S/2}$ = population with half sensitivity
i.e. requiring 2 units of drug for 50% kill
$P_{S/Z}$ = populations with Z level of partial resistance
i.e. requiring Z units of drug for 50% kill

These definitions permit the quantitatively accurate depiction of tumour kill and regrowth to be illustrated.

We now consider the effect of a series of doses (Fig.2). The first dose does not have an "intensity" and yields an immediate kill; subsequent doses have their net effect reduced by regrowth during the treatment interval i.e. the net kill slope reflects dose intensity. In passing it may be noted that the first dose of a treatment program is determined solely by the protocol, but the subsequent intensity is usually determined by titrating the dose or interval to the chosen level of toxicity.

The net kill "nadir" (Skipper, 1988) after **n** cycles of dose **u** at interval **i** (in doubling times t_d) is given by:

$$\underset{\log_2}{\text{Net Kill}} = \underset{n \times u}{\text{Total Dose}} - \underset{(n-1) \times i}{\text{Rx Duration}} \qquad \ldots(\text{Eq.}1)$$

This is a normalized version of the Lloyd formula (Skipper and Schabel, 1982).

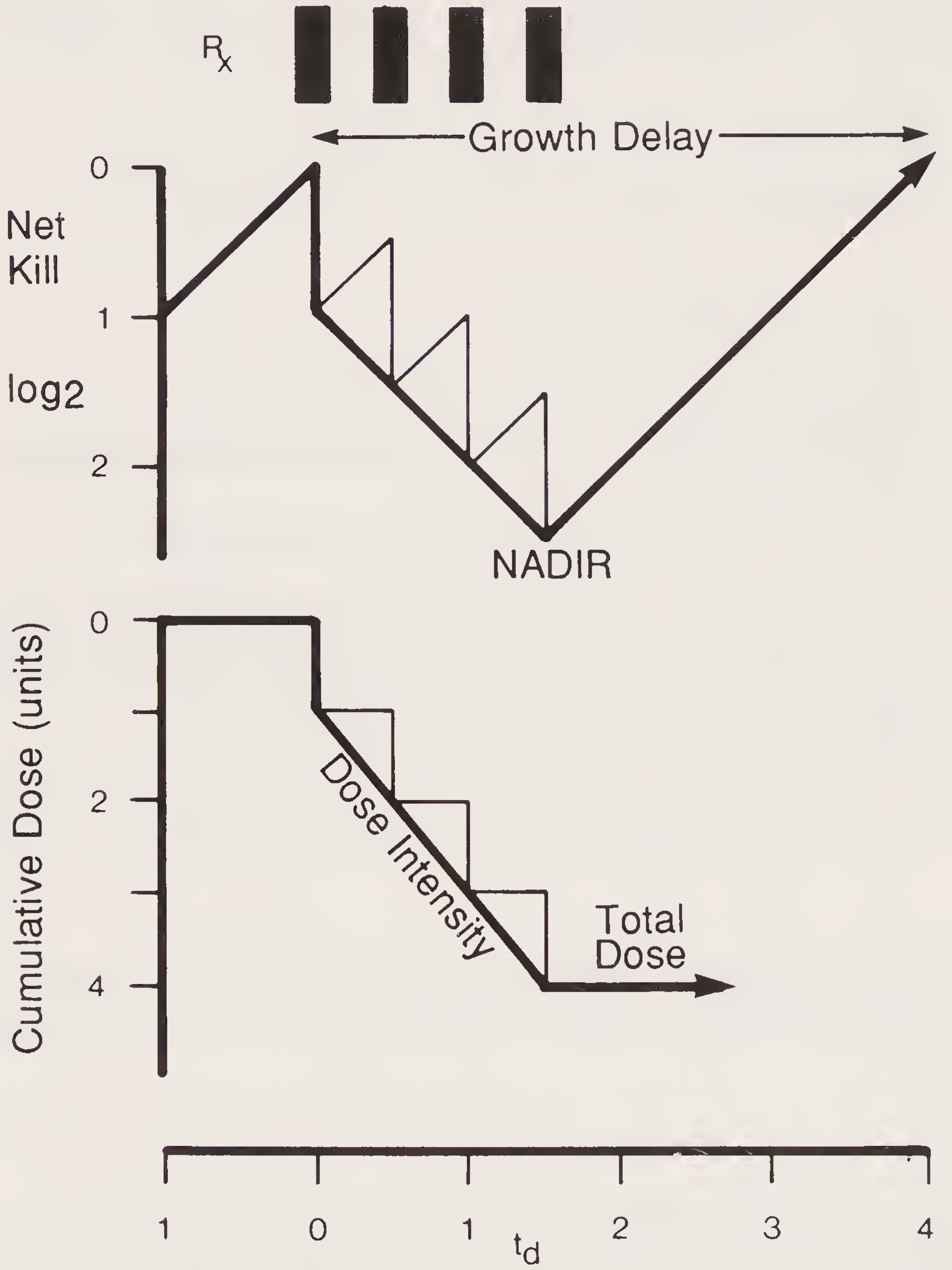

Figure 2. Diagram of the effect of four doses of one unit (each causing a 50% cell kill) given every half doubling time. The thick line represents the net cell kill (above) or cumulative dose (below). The net kill slope reflects dose intensity, but growth delay depends only on total dose (4 units of dose causes 4 t_d delay).

For the example in Fig.2:

Net Kill = $\log_2$ kill - regrowth doublings during therapy
= drug units - duration of therapy
= 4 - 1.5 = 2.5 ($\log_2$).

Because of the way in which dose is measured:

Growth Delay (in multiples of t_d) = Total Dose (units) .....(Eq.2)

Conversely, if growth delay and doubling time are known, then the net kill can be calculated.

These relationships are explored graphically in Fig.3, a normalized conversion of Skipper's description of the chemotherapy response of the L1210 system for which the assumptions of the present simplified model appear to fit well (Skipper, 1988). A dose size is required for illustration but affects only the initial step and the slope of the line connecting the step and the nadirs: the nadirs and total dose lines are unaffected by choosing a different dose.

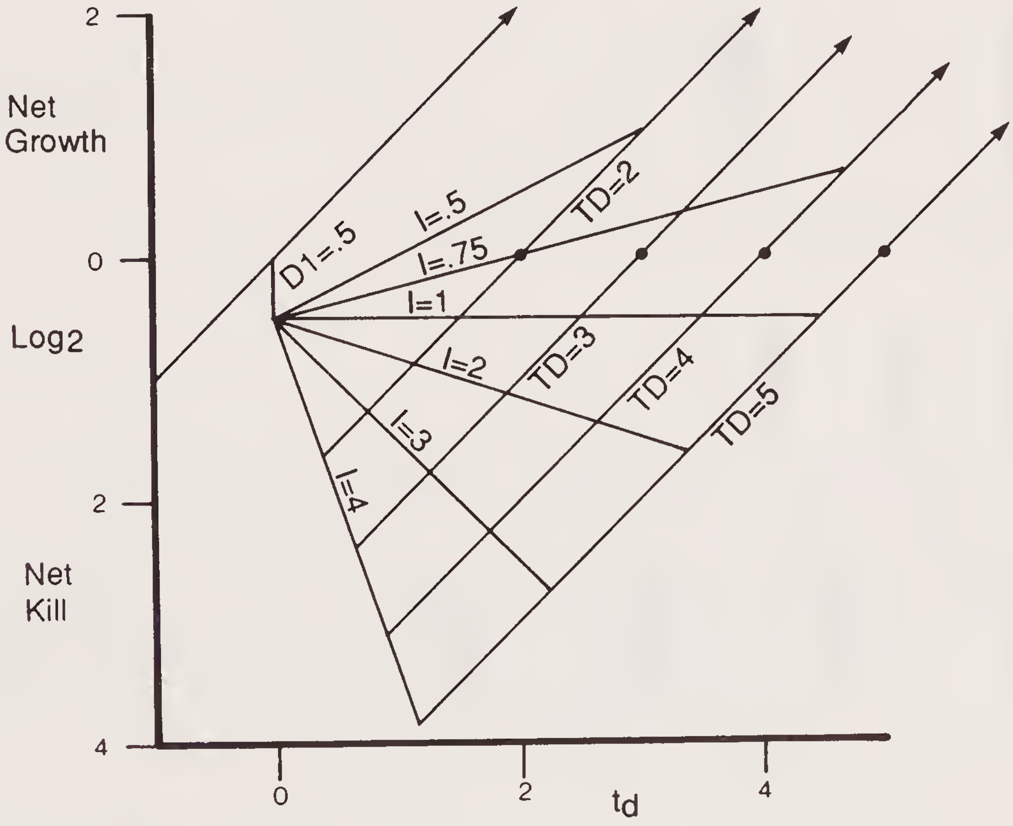

Figure 3. A series of 0.5 unit doses D_1-D_n are given at normalized dose intensities I=0.5 to I=4 units per t_d, for total doses TD of 2-5 units. The kill-regrowth steps have been omitted for clarity.

PARAMETER ISOLATION

Before proceeding with further refinements of the model, consider the approach to the isolation of the three parameters: dose size u, total dose TD, and dose intensity I, illustrated in Fig.4. The dose-response curve can be evaluated by comparing small frequent doses with large less frequent ones, maintaining unchanged the dose intensity and the total dose. Total dose effect is isolated by adding extra standard cycles.

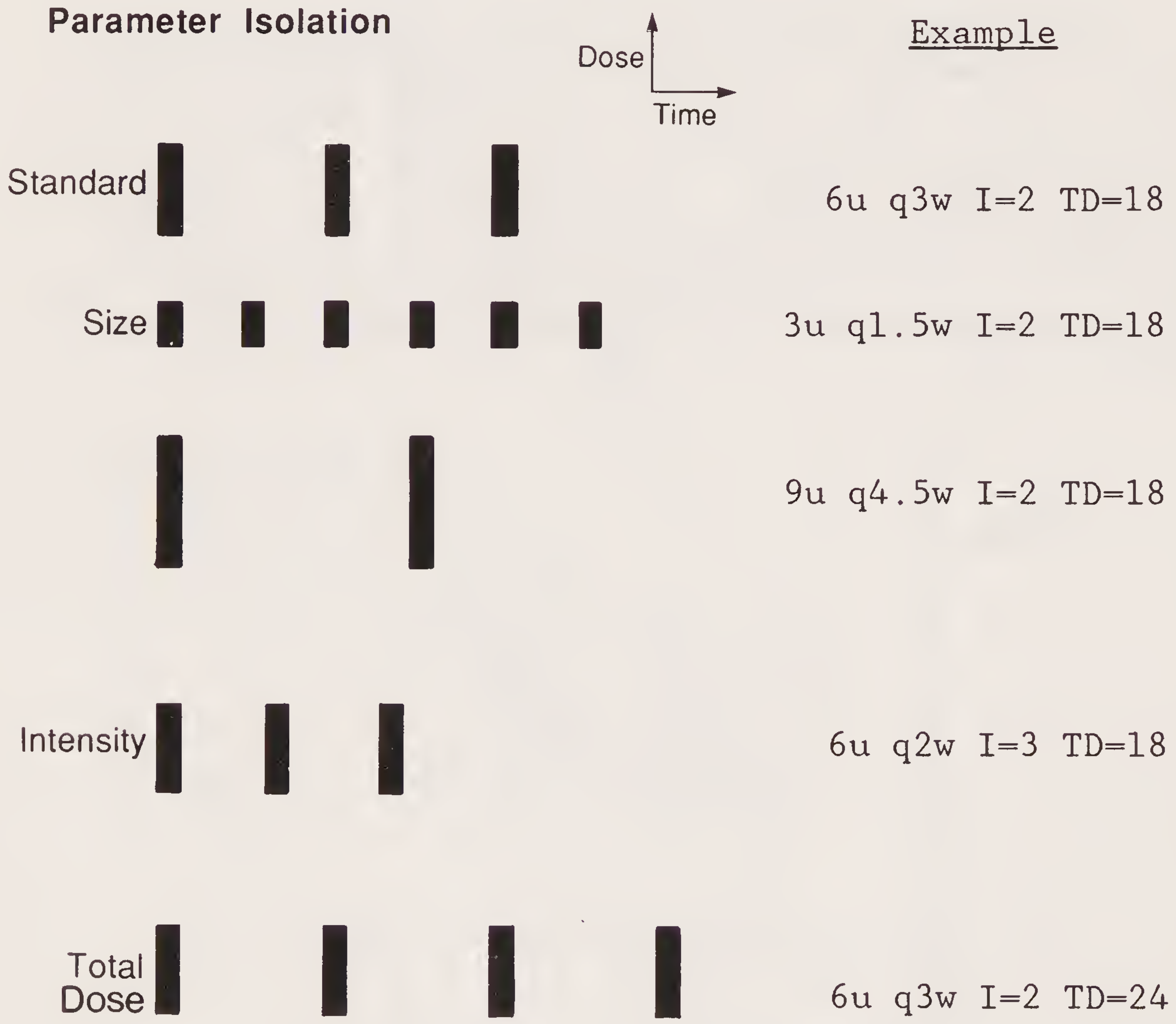

Figure 4. In comparison to a standard program (top), the protocol can be varied in a way which isolates the parameter listed in the the left column whilst keeping the other two parameters constant.

Any independent effect of dose intensity should be evaluated by adjusting dose frequency rather than dose size unless the dose-response relation is known to be linear. To illustrate this approach in the context of the simplified model (Fig.5), protocol A in which three doses of 1.5 u are given at intervals of 1 t_d (intensity I_A=1.5 u per t_d) is compared with protocol B given at half the interval (I_B=3). Intensity is predicted to affect the net kill but not the growth delay (in non-curative situations).

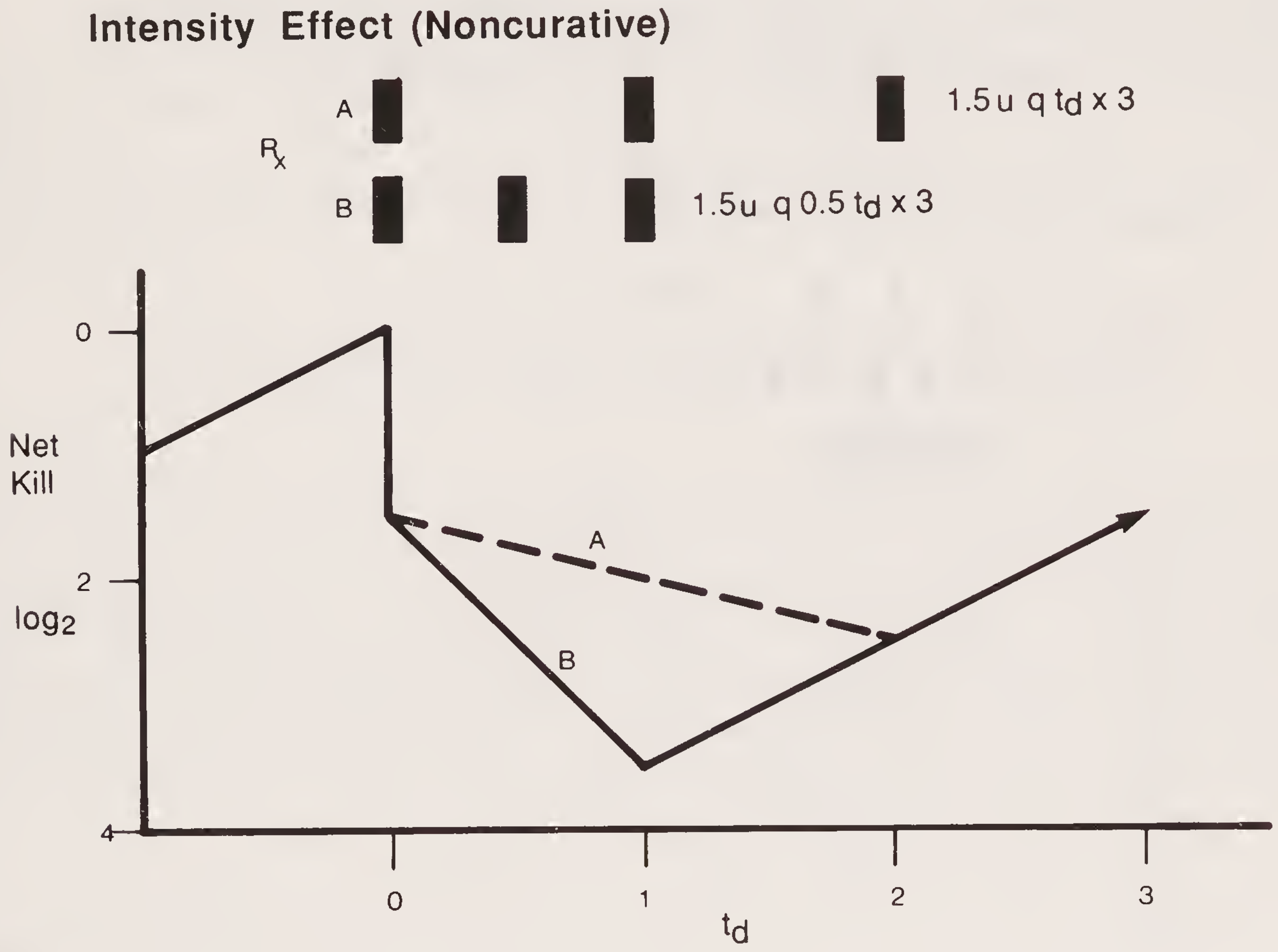

Figure 5. Protocol B (solid line) has twice the dose intensity of A (dashed line) resulting in a greater net cell kill ($2^{3.5}$ vs $2^{2.5}$ respectively). On completion, they both resume the same regrowth line yielding a growth delay of 4.5 t_d (= the total dose in standard units).

LIMITS OF MODEL PREDICTIONS

The relationships described by equations 1 and 2 can be expected to break down once the sensitive cell population has been eliminated. This will occur in two dissimilar situations: a) resistant cells absent (system curable), or b) resistant cells present (system may or may not be curable depending whether the resistance is partial or absolute).

No Drug Resistant Cells

Elimination of the sensitive cell population in the absence of drug resistance results in cure and any additional doses are redundant. However treatment intensity will determine the amount of drug required to eliminate the tumour (Fig.6). The relationship

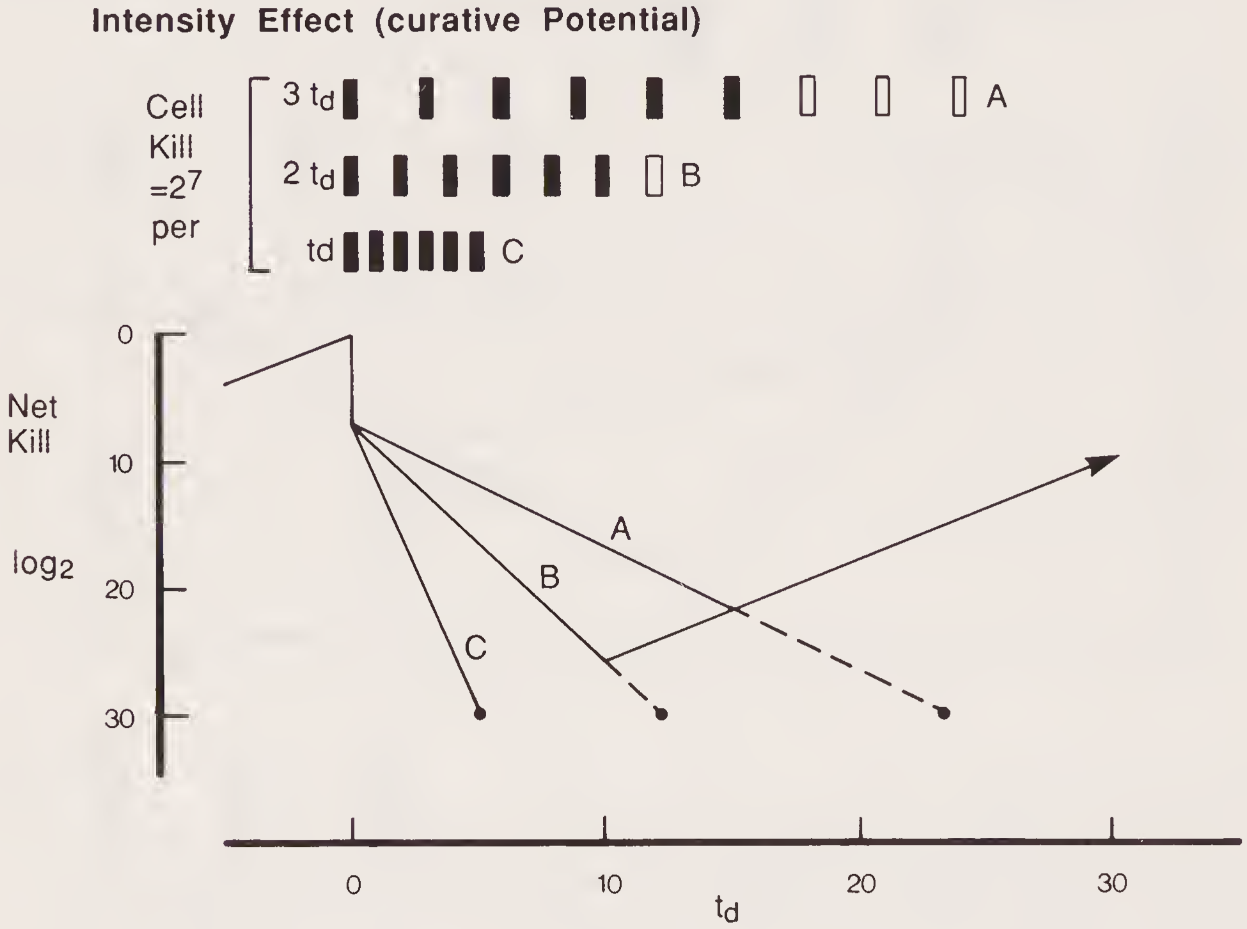

Figure 6. Three programs are applied to a uniformly sensitive population of 2^{30} cells ($\sim 10^9$), each using a dose size of 7u (i.e. each dose produces a $2^7=128$-fold kill). The most intense protocol C gives one dose every doubling time and eliminates the tumour after 6 doses. Protocol B treats every $2t_d$ and protocol A every $3t_d$; if stopped after 6 doses, residual tumour would adopt the same regrowth line. Residual cells could be eliminated with a further 1-3 cycles (open boxes). Note the total duration of A is 4.8 ($8_x 3/5$) times as long as protocol C.

between intensity and curative total dose is a rectangular hyperbola with asymptotes of a minimum intensity of 1 dose unit per t_d, and a minimum total dose which would have to be given as a single high dose treatment. The same considerations apply to other degrees of net cell kill such as those required to produce a partial or complete clinical response.

Resistant Cells Present

Suppose that the tumour consists of a mixture of sensitive and totally resistant populations of cells P_S and P_R, assumed to have the same doubling time (Fig.7, thick lines). Then the maximum possible growth delay is determined by the ratio of the two groups at the start of treatment:

$$\text{Maximum growth delay (in doubling times)} = \log_2[(P_S+P_R)/P_R]$$

At the point where the number of sensitive cells has been suppressed to equal the number of resistant cells (Fig.7, point a), only one further t_d growth delay is possible. From equation 2, the total dose to reach the crossover (point a) is also equal to the population ratio, and for practical purposes this may be regarded as the maximum useful total dose.

Partial Resistance

A state of partial resistance is equivalent to a smaller cell kill for a given dose of drug. Thus a population $P_{S/2}$ would experience only half the kill of population P_S (Fig.7). This discussion will consider only the combination of partial and total resistance (partial resistance in a curable tumour will determine minimum curative intensity). The potential significance of these partially resistant minorities depends on their relative frequency compared to the sensitive and completely resistant cell clones. Populations with a Z degree of drug resistance are denoted by $P_{S/Z}$ (Table 1).

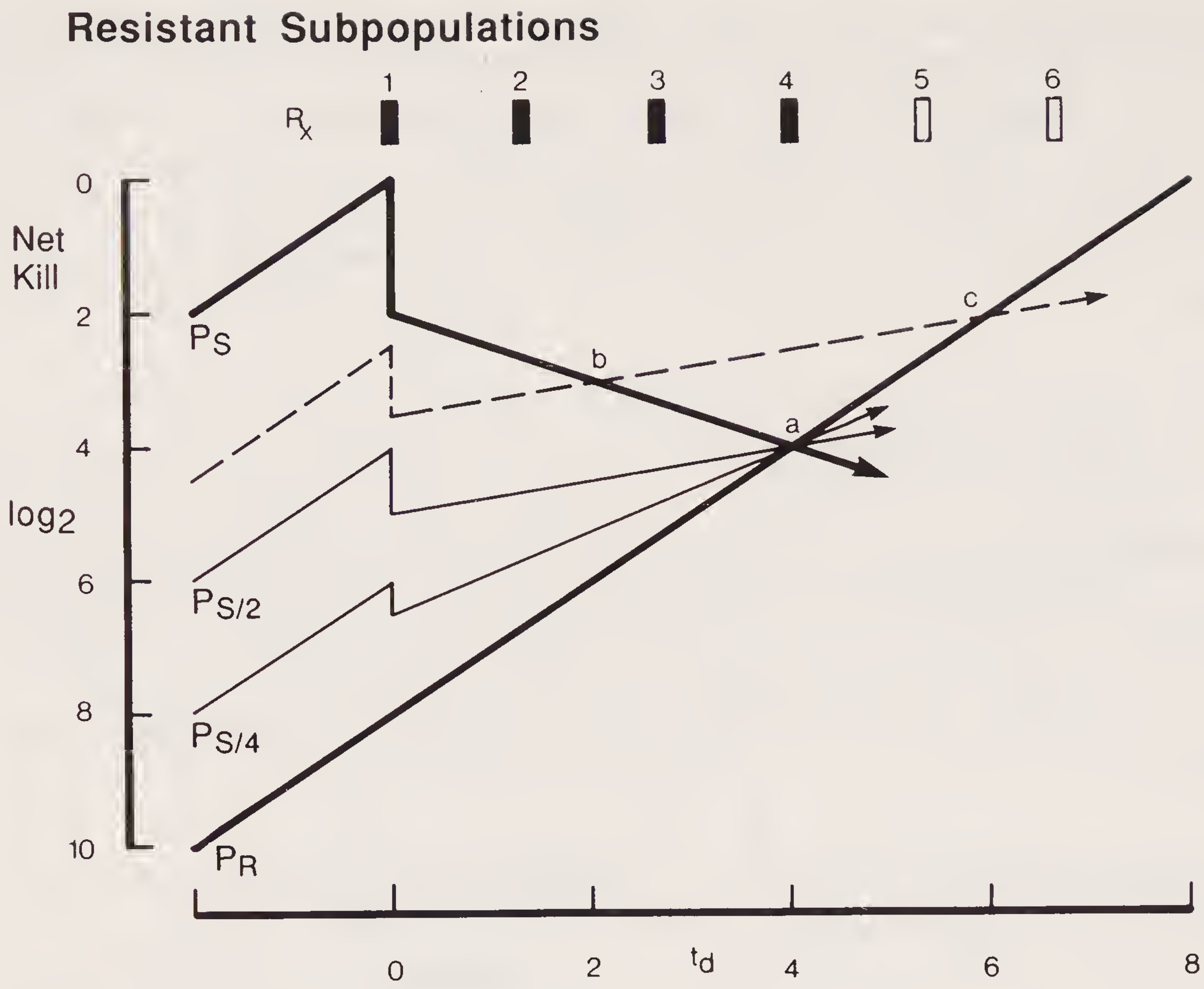

Figure 7. Drug treatment Rx (2u q1.3t_d) yields a net positive kill on the sensitive population P_S until the resistant population P_R is encountered (point a). The effect of partially resistant populations $P_{S/2}$ and $P_{S/4}$ is described in the text.

If the initial population of each of the tumour clones satisfies:

$$\log_2 P_{S/Z} = \log_2 P_R + (\log_2 P_S/P_R)/Z$$

as shown for Z=2 and Z=4 in Fig.7, then under therapy $P_{S/Z}$ will become equal to the number of absolutely resistant cells P_R at the same point for each clone (point a). If the proportion of partially resistant cells is equal to or less than this value, their presence will be obscured by P_R. However, if their proportion is greater as shown for $P_{S/2}$ (Z=2) by the dashed line, then apparent resistance will appear earlier (point b). Additional drug can still lead to further growth delay until the completely resistant population becomes numerically dominant (point c) beyond which suppression of the other clones has little effect.

The dose intensity of a protocol determines the degree of resistance that would still allow a positive net cell kill. Thus Protocol A (Fig.8) would appear to encounter drug resistance after only 8u of drug and might be terminated prematurely compared to the more intensive Protocol B. Note in this example, however, that the total obtainable growth delay for either protocol is determined by the absolutely resistant population P_R encountered after 16u of drug.

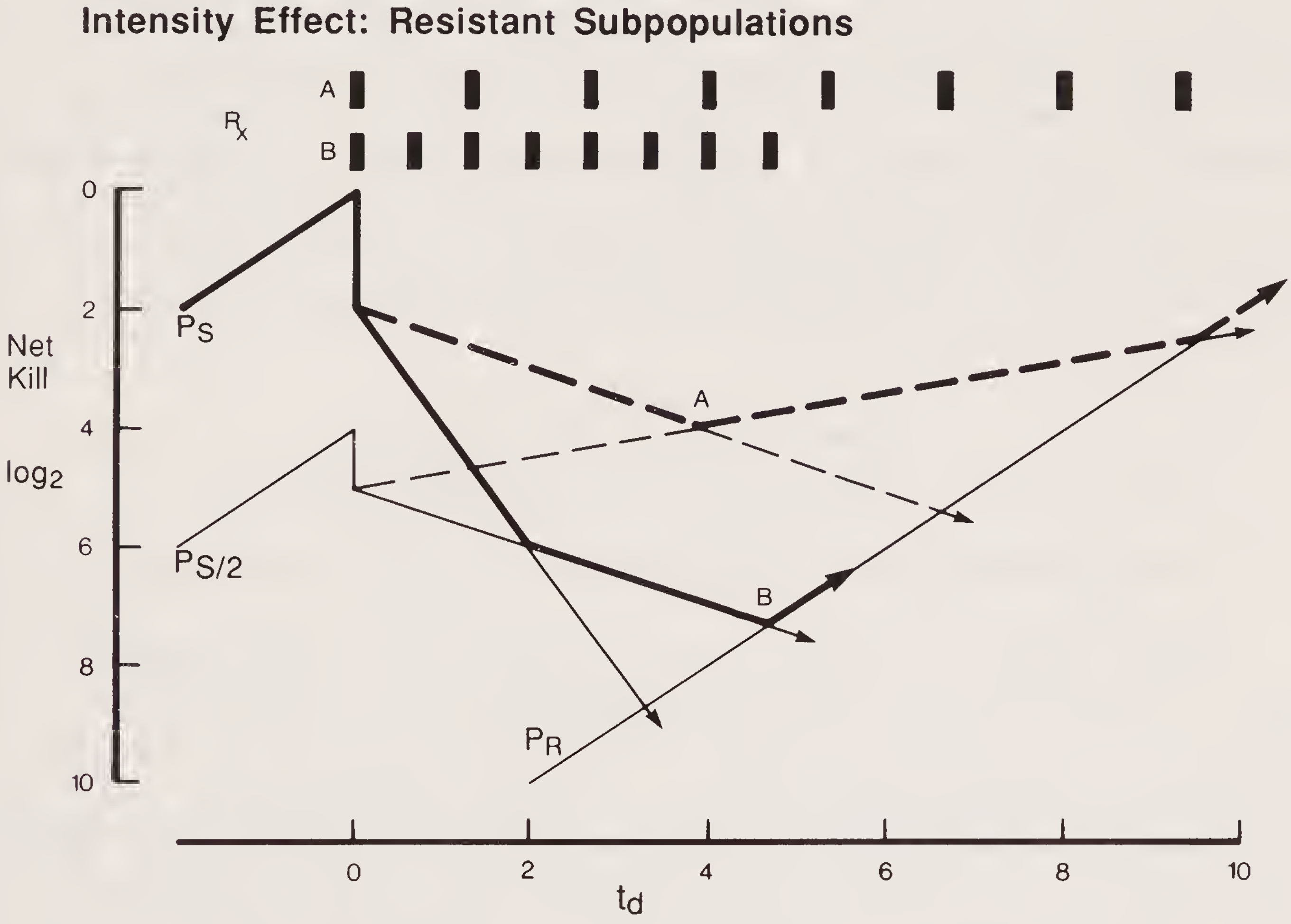

Figure 8. Protocol A (same as Fig.7) is compared with protocol B with twice the dose intensity which is able to yield a net cell kill on a population $P_{S/2}$ only half as sensitive as P_S. Protocol A appears to encounter early failure though maximum growth delay is determined by P_R.

CLINICAL CORRELATES

In the clinic, we are not generally in a position to measure directly the proportion of lethally damaged cells resulting from a chemotherapy treatment. There is generally a considerable delay before kill is manifest as a change in tumour size, with a marked damping effect on the phenomena described for the model situation. However, there are some relevent measurements that might provide a basis for comparison. These include untreated doubling time, growth delay, and possibly cell kill nadir. Growth delay resembles clinical time to progression, but requires an adjustment for any difference in tumour size between initiation of treatment and detection of progression; rarely will it be ethical to wait until regrowth to the initial state before adopting other treatment measures. Tumour markers could be used as relatively responsive measurement tools. Some estimate of cell kill nadir may be obtained from the degree of gross reduction of tumour size or tumour marker level, but in general this will be an underestimate. Where the growth delay and the doubling time are known, cell kill nadir can be calculated from combining equations 1 and 2:

$$\underset{\log_2}{\text{Net Kill}} = \underset{(\text{in } t_d)}{\text{Growth Delay}} - \underset{(\text{in } t_d)}{\text{Rx Duration}} \qquad \text{......(Eq.3)}$$

for therapy given while the tumour is fully sensitive.

The cumulative dose plot of delivered treatment (Fig.2, below) is a convenient surrogate for cell kill, providing information on both delivered intensity (slope) and total dose (Coppin, 1987). In the absence of direct measurement of kill nadir or growth delay, cumulative dose comparisons may provide some insight into the relative impact of different protocols using the same drugs.

CONCLUSIONS

The use of a simple model of chemotherapy effect suggests the following relationships:

1. Growth delay is a function of total dose, not intensity.

2. Dose intensity determines the amount of drug required to achieve a given net cell kill.
3. Dose intensity determines the degree of partial drug resistance compatible with net cell kill.

In the clinical setting, total dose is predicted to correlate with remission duration whereas remission rate should correlate more closely with dose intensity. In palliative situations, the dose intensity on this model would only need to be sufficient to suppress the tumour sufficiently to relieve symptoms. High dose intensity becomes much more critical if cure is a realistic objective though adequate total drug must still be given.

The phenomenon of emergence of a drug resistant clone during ongoing chemotherapy for advanced cancer is well recognized in everyday clinical practice, and the program is generally discontinued at that point. In those situations where no good alternative therapy is available, the possibility of partial resistance should be given consideration: the dose intensity of the program might be escalated if practicable, otherwise continued growth restraint may still be achievable until absolute resistance is encountered.

The strong retrospective correlation between delivered dose intensity and outcome measures deserves comment. In the advanced disease setting, treatment delivery may be dependent on patient variables, such as performance status or compromise of dose limiting organs, which may be the more proximal determinant of outcome. In the adjuvant setting, protocol design has generally consisted of a fixed number of cycles irrespective of dose reductions or delays, resulting in an inevitable close correlation of dose intensity with total dose. Even current prospective studies attempting to evaluate the independent effect of dose intensity have tended to adjust dose size rather than dose frequency, and their conclusions will be difficult to interpret because of the uncertain role of dose-response as well as dose intensity. Prospective studies which examine dose frequency as the only variable are recommended.

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COMMENTS ON KINETICS AND BIOLOGY OF THE RESIDUAL CANCER, AND ON RELEVANT THERAPEUTIC STRATEGIES BASED ON THESE PHENOMENA

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INTRODUCTION:

Effects of therapeutic perturbation on the behavior of malignancy surviving therapy - the main theme of this Symposium - have not been adequately studied in human tumors. In experimental tumors, however, ample evidence indicates profound influence of therapeutic intervention on tumor kinetics and on the behavior of metastases (Clunet, 1910; Tyzzer, 1913; Delarue, 1960; Drye et al., 1962; De Wyss, 1972; Simpson-Herren et al., 1976; Gorelik, 1978; Gunduz et al., 1979; Fisher et al., 1983; Fisher et al., 1989). Many aspects of this sensitive interaction in the clinical setting are virtually unresearched, yet the effects of noncurative therapy on tumor kinetics and biology may be of great relevance, affecting significantly the subsequent behavior of the disease.

There should be concern with noncurative procedures, as the extent of their final side effects is far from appreciated. If found, as expected, to impact the system deleteriously, then procedures such as open biopsies, definitive surgery and also many instances of noncurative chemotherapy and radiotherapy will all have to be scrutinized thoroughly, as virtually every patient may be affected. To minimize the adverse effects, the frequency and extent of noncurative procedures should be kept at minimum. In cases where some degree of cytoreduction is unavoidable, therapeutic strategies aimed at the protection of the host from the effects of cytoreduction should be declared high priority research.

This report will summarize experimental and clinical data relating to what is known about the consequences of therapeutic intervention on the residual tumor, and will attempt to explain some of the biological and pathophysiological mechanisms of these observations.

Also, a brief review of clinical strategies aimed to minimize those clinically relevant spinoffs of therapy will be provided.

NONCURATIVE SURGERY AND TUMOR KINETICS - ANIMAL STUDIES

As seen in Table I, already at the turn of the century, Clunet documented, in a non-metastasing mouse model, an increased incidence of systemic metastases after the resection of the primary tumor (Clunet, 1910). Tyzzer, summarizing Clunet‘s experiments, discussed the concept of athrepsia, i.e. a competition for overall nutrition in the tumor system, as a main explanation for this phenomenon (Tyzzer, 1913).

In his own experiments with murine lung models he documented that mice with resected primary tumors had more lung metastases, and of larger size, than non-operated animals. Incomplete excision of the tumor resulted in an increase of metastases in a fashion similar to a complete excision. Also, massage of the tumor 18 and 21 days after tumor inoculation led, on day 36, to a significantly increased number of lung metastases compared to non-massaged animals. Massage and excision resulted in more lung metastases than seen either with massage or excision alone (Tyzzer, 1913).

These experiments indicated that perisurgical tumor manipulation, with or without cytoreduction, may play an important role in subsequent evolution of the disease. The experiments also suggested that, in addition to the pre-formed metastases present before diagnosis and surgery, a certain proportion of metastases may be released during, and as a result, of the surgical procedure. As seen in tables II and III, the next several decades confirmed these observations (De Wyss, 1972; Simpson-Herren et al., 1976, Gorelik, 1978; Gunduz et al., 1979; Fisher et al., 1983; Fisher et al., 1989).

Gorelik concluded that the inhibition of metastases by the primary tumor is tumor specific - melanoma lung metastases were inhibited only by melanoma reinoculation and not by Lewis's lung carcinoma, and the other way round (Gorelik et al., 1978). Inhibition was also size dependent: the larger the volume of reinoculation, the more effective the inhibition of metastases. Reinoculation of irradiated cells (decreased viability and metastatic potential) failed to inhibit metastases (Gorelik,1978). Simpson-Herren et al., using Lewis lung tumor model, showed a significant increase in proliferation after cytoreduction (Simpson-Herren et al., 1976). Their experiments suggested that the increase of metastatic proliferation after cytoreduction was a likely consequence of kinetic acceleration of preexisting, previously dormant metastases.

TABLE I

ASSOCIATION OF SURGICAL CYTOREDUCTION AND BEHAVIOR OF KINETICS - ANIMAL EXPERIMENTS, EARLY DATA

PROCEDURE	RESULT (Compared to Controls without Procedure)	REFERENCE
Resection of the Primary Tumor	↑ Incidence of Systemic Metastases	Clunet, 1910
Resection and/or Massage of the Primary Tumor	↑ Quantity of Systemic Metastases	Tyzzer, 1913
	↑ Size of Lung Metastases	Tyzzer, 1913

TABLE II

EXPERIMENTAL STUDIES: EFFECTS OF THE PRIMARY TUMOR ON THE GROWING FRACTION OF THE RESIDUAL TUMOR - 1972 - 1976.

PHENOMENON- PRIMARY TUMOR	RESULT	REFERENCE
Growth of Primary Tumor → ↓ Elaboration of Inhibitory Substances ↗	Reduced Rate of Cell Division In: -Primary Tumor -Metastases	De Wyss, 1972
Cytoreductive surgery, and/or Sham surgery	↑ Growth Fraction of Systemic Metastases	Simpson-Herren 1976

De Wyss confirmed a progressive retardation of the growth rate of the primary tumor and its metastases (Table II). In addition, he documented the presence of inhibitory substances that increased in parallel with tumor growth (De Wyss, 1972). These inhibitory substances, elaborated by the tumor, are systemic in nature and of two kinds: non-specific, where inhibition of the cellular growth is not limited to the tumor but affects equally the cells of the host (primary reason for the loss of weight of the host); and tumor tissue-specific, where only the tumor is inhibited.

The above mentioned experimental studies have shown an association between the changes in the tumor cell kinetics and therapeutic interventions, in particular with noncurative surgical cytoreduction. More recently, Fisher et al. confirmed their original observations (Gunduz et al., 1979; Fisher et al., 1983) of an increased growth fraction after cytoreductive surgery suggesting a direct recruitment of cells from G0 into G1 and S phases after the noncurative cytoreductive procedure (Fisher et al., 1989). The increase of the growth fraction is likely a consequence of several mechanisms activating cells into the cycle. In their most recent paper, they documented the presence of a mitogenic growth factor in the serum of animals undergoing noncurative surgery (Fisher et al., 1989). Not only did animals undergoing such surgery exhibit an increase of the labelling index in the distant tumor foci, but also a transfer of their serum resulted in the stimulation of the labelling index of tumors in animals not undergoing surgery (Table III). Furthermore, preoperative treatment with cyclophosphamide, radiotherapy and also tamoxifen and zoladex, prevented rise in the labelling index.

These experiments provide a strong evidence favoring the systemic effects of incomplete surgery affecting the behavior of the distant residual disease, and support the data claiming the presence of growth factors mediating the tumor progression and the fluctuation of its growth. As discussed below, the source of growth factors likely implicates both tumor, as well as nonmalignant tissues. Fisher's results also confirm the expected benefit of preoperative treatment abrogating kinetic alterations after cytoreduction (Fisher et al., 1989).

Elsewhere in this volume, Braunschweiger discusses not only alteration of kinetics after cytoreduction, but also significant alterations in tumor neovascularization postoperatively. The importance of these observations cannot be underestimated, particularly as enhancement of intravascular dissemination postsurgically, and the overall delivery of chemotherapy, may all be affected. As discussed by several workers, the alteration of the growth rate after surgery has been observed not only in tumors, but also in nonmalignant tissues, with the liver and kidney as prime examples (Weiss, 1980; Cameron, 1983; Stiles, 1985; Polak, 1989; Fidler, 1989).

TABLE III

EXPERIMENTAL STUDIES; EFFECTS OF THE PRIMARY TUMOR ON THE GROWTH FRACTION OF THE RESIDUAL TUMOR - 1978 - 1989

PRIMARY TUMOR	RESULT	REFERENCE
Re-Inoculation of Live Melanoma cells	↓ Growth Rate of Melanoma (not lung) Metastases	Gorelik,1978
Reinoculation of Irradiated Melanoma Cells	No Reduction of Growth Rate of Melanoma Metastases	Gorelik,1978
Resection of the Primary Tumor	↑ Growth Fraction of Lung Metastases GO → G1	Gunduz et al 1979 Fisher et al 1983
Transfer of Serum from Animals with Resected 1-ARY Tumor	↑ Growth Fraction of Metastases in Animals without Resected 1-ARY Tumor	Fisher et al 1989

KINETICS - HUMAN STUDIES

If the enhancement of dormant metastases by noncurative therapeutic intervention is a true phenomenon in animals, it may, similarly, apply to human tumors. The evidence is not as abundant in a clinical setting, and fewer studies in this regard are available. As well, animal tumors are known to be kinetically and antigenically easier to manipulate, and to be more homogeneous than human tumors. Nevertheless, some documented evidence of kinetic association with cytoreduction and tissue trauma, as indicated in multiple anecdotal reports, is available in human tumors.

TABLE IV

HUMAN STUDIES - ASSOCIATION OF SURGICAL CYTOREDUCTION AND BEHAVIOR OF METASTASES

PHENOMENON	RESULT	REFERENCE
Presence of Circulating Cancer cells	↓ Survival	Roberts, 1961 Watne, 1960
	↑ Recurrence Rate	Drye, 1971
2nd Look Laparotomy - (Testicular Tumors)	↑ Values of Markers (A-fetoprotein B-HCG)	Lange, 1980

Delarue summarized in his review the incidence of increased locoregional and systemic perisurgical spread of malignant cells in patients undergoing surgical treatment for newly diagnosed malignancy (Delarue, 1960). The perisurgical vascular spread was documented by an increase of circulating cancer cells in the blood stream. Other workers confirmed some of these observations, reporting the presence of circulating cancer cells 7 to 9 days after surgery with the yield of circulating cells corresponding to the tumor stage and other tumor characteristics (Drye, 1972; Roberts et al., 1961; Watne, 1960).

Although several workers found no clear association between circulating cancer cells and subsequent survival in clinical setting, there are some data confirming it (Table IV). Roberts et al. reported that patients exhibiting circulating tumor cells during surgery had a significantly lower 5 year survival compared with patients with no circulating cancer cells (Roberts, 1961). Similarly, Watne reported improved 18 month survival in patients without circulating cancer cells (Watne, 1960), and Drye reported higher recurrence rate in patients with positive circulating cancer cells (Drye, 1972). Therefore, it appears plausible that although the majority of circulating cells fail to establish metastases, a subset of metastases with increased metastatic potential may successfully metastasize during the postsurgical time period. In their work, Fidler et al. confirmed the existence of subsets of metastases with a low and a high metastatic potential (Fidler et al., 1984). It is more than likely that increased acceleration and kinetic boost in connection with cytoreduction may affect more profoundly the kinetically more active and biologically more aggressive subset of metastases. Thus, the noncurative cytoreduction may affect preferentially the pre-existing clones of cells with high metastatic potential rather than their more benign counterparts.

Fluctuation of serum markers (alphafetoproteins and serum beta-HCG) in testicular tumors after surgery was reported by Lange who confirmed a temporary increase of tumor growth during the post-surgical time period (Lange, 1980).

KINETICS AND METASTATIC POTENTIAL

Is there any evidence that acceleration of kinetics, with or without cytoreductive procedure, will enhance the metastatic potential of the residual tumor? If so, what are the mechanisms of such phenomena? There are data indicating that during, or soon after cell division, both the cell detachment and transvascular diapedis are increased (Weiss, 1980). Although not fully researched, such a constellation of events leading to enforcement of metastases into the circulation, is scientifically very acceptable, and fits the postulation that it is at the time of the cell division rather than during the metabolically inactive, non-dividing stage, that the cell motility is enhanced and that the biochemical apparatus mediating metastatic activity takes place.

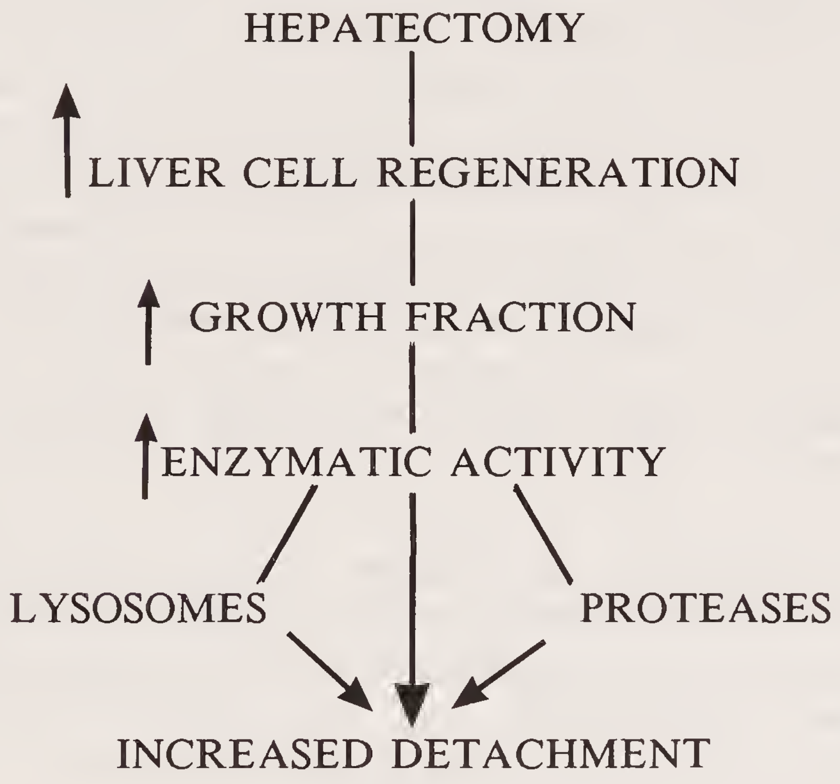
Weiss reported, in his in-vitro data, that increasing growth rate facilitated cell detachment from solid surfaces, and in-vitro cells commonly detached during specific times of cell cycle, especially at metaphase (Weiss, 1978; Weiss, 1980).

GROWTH FRACTION AND CELL DETACHMENT

In an attempt to determine the correlation of non-specific tissue growth and cell separation, Weiss studied effects of partial hepatectomy on cell detachment (Table V). Within the second postoperative day following partial hepatectomy, increased hepatic regeneration was observed, as documented by increased liver weight, protein content and mitotic index. Associated with these changes, significantly more cells were detached from the partially hepatectomized animals than from the sham-operated groups, indicating not only that the intrinsically higher proliferation rate, but also the induction of proliferation by surgery leads to increased cell detachment (Weiss, 1980).

TABLE V

PATHOLOGY OF CELL-TO-CELL CONTACT



Other data indicate that additional factors facilitating the cell release in connection with increased cell division include increased enzymatic activity, such as lysosomes and other proteases. Hence, the biochemical humoral nature of the cell contact and detachment, including action of substances such as growth factors, or enzymes, plays a significant role in the propagation of tumor spread and altered biology of metastases. Furthermore, enhancement of tumor and liver cell detachment was seen prior to the incubation of these cells with necrotic tissue extracts, or enzymes from local necrotic regions of the tumor and degenerating macrophages (Weiss, 1977). In this context, postoperative necrotic tissues may assist in tumor dissemination after the surgery, before the removal of necrotic tissues by the RES system is complete.

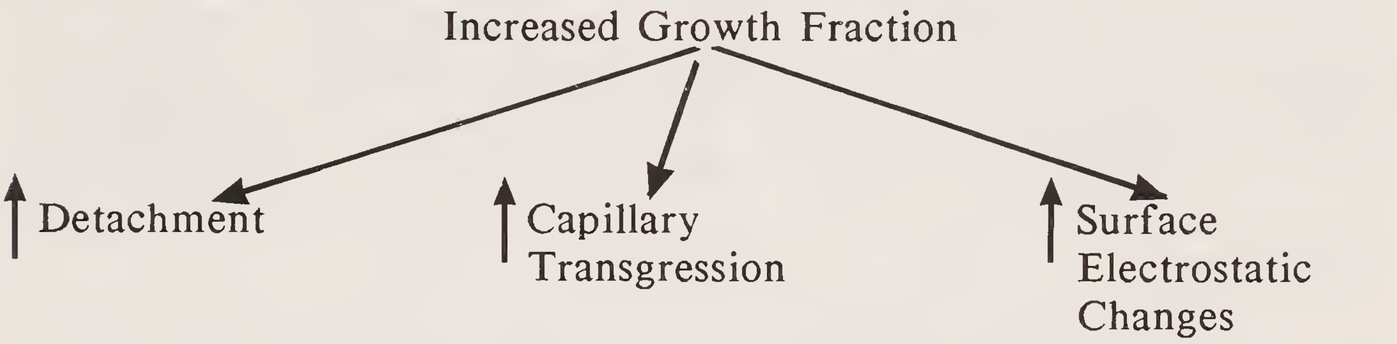
Further association of tumor growth and increased cell detachment was seen after the addition of medium nutrients and other factors enhancing growth in in-vitro cell cultures (Weiss, 1980).

These experiments were subsequently expanded to determine if cell surface electrostatic charges could play a role in cell contact and adhesions. It was observed that when the growth rates of cells in suspension culture were reduced by medium depletion, or increased by medium replenishment, their net surface electrostatic potentials, with subsequent alteration of cell adhesion, were correspondingly reduced or increased. Also, alterations of enzyme associated anionic sites, likely mediating the electrostatic changes of the cell surface, and linking enzymatic activity and electrostatic potential, were documented in rapidly proliferating, but not in the resting lymphoid cells (Weiss, 1980).

Contact mediation of adhesion and detachment of tumor cells by proteins such as fibronectin was documented by Stenman et al., and also summarized by Weiss (Stenman et al., 1977; Weiss, 1980). Stenman showed increase concentration of fibronectin on the cell surface during the non-dividing parts of cell cycles (during G1, S and G2), but its reduction in cells undergoing mitosis.

TABLE VI

CORRELATION OF TUMOR GROWTH FRACTION AND PATHOLOGY OF METASTATIC INVASIVENESS (ACCORDING TO WEISS, 1980)


Increased Growth Fraction
↑ Detachment
↑ Capillary Transgression
↑ Surface Electrostatic Changes

These experiments provide some evidence for an association of increased cell division and increased invasiveness which could conceivably be linked with enhanced perioperative or early postoperative cell dissemination (table VI). While access of the tumor cells to the vasculature, and subsequent survival of metastases in the circulation and tissues, is a complex process where intrinsic tumor cell characteristics likely play a dominant role, (Fidler et al., 1984) the environment of the host, particularly the fluctuation of stimulatory or inhibitory growth factors generated by the host, may modify significantly the establishment of metastases (Fidler, 1989).

In the case of increased kinetics after cytoreduction, all evidence indicates that these alterations may lead to a wide array of additional behavioral changes resulting in the enhancement of metastatic formation. Recognition of these mechanisms is the first step in our understanding of some aspects of the behavior of metastases leading towards the development of successful therapeutic interventions.

GROWTH FACTORS

GROWTH FACTORS AND CYTOREDUCTION

According to animal studies, postsurgical kinetic alterations in the surviving tumor start within minutes after surgery, and may last for several days (De Wyss, 1972; Simpson-Herren et al., 1976; Gunduz et al., 1979; Fisher et al., 1983; Fisher et al. 1989). While the mechanism for the cytoreductive kinetic kick is complex and not adequately explored, association with stimulatory and inhibitory growth factors has been postulated by several workers. As discussed above, more than a decade ago De Wyss indicated the presence of an inhibitory substance produced by the tumor (De Wyss, 1972). Partial debulking of the tumor, and a reduction of the levels of these inhibitory substances, may explain the subsequently documented tumor growth stimulation. Similar conclusions were reached by Gorelik et al. (Gorelik et al., 1978); and most recently, confirmed by Fisher et al., (Fisher et al, 1989). Recent recovery of stimulatory and inhibitory growth factors active under physiological conditions such as embryogenesis, early tissue growth, and tissue trauma, has been recently documented and described independently by several laboratories (Table VII). These factors include stimulatory substances such as platelet derived growth factor, PDGF (Antoniades et al., 1979), transforming growth factor alfa, TGF-A (Derynck, 1987), epidermal growth factor, EGF (Fitzpatrick et al., 1984), insulin-like growth factors, IGF-I, IGF-II (Baxter, 1986), transforming growth factor beta, TGF-B (Sporn, 1986), etc.

TABLE VII

ORIGIN OF GROWTH FACTORS - SCHEMA

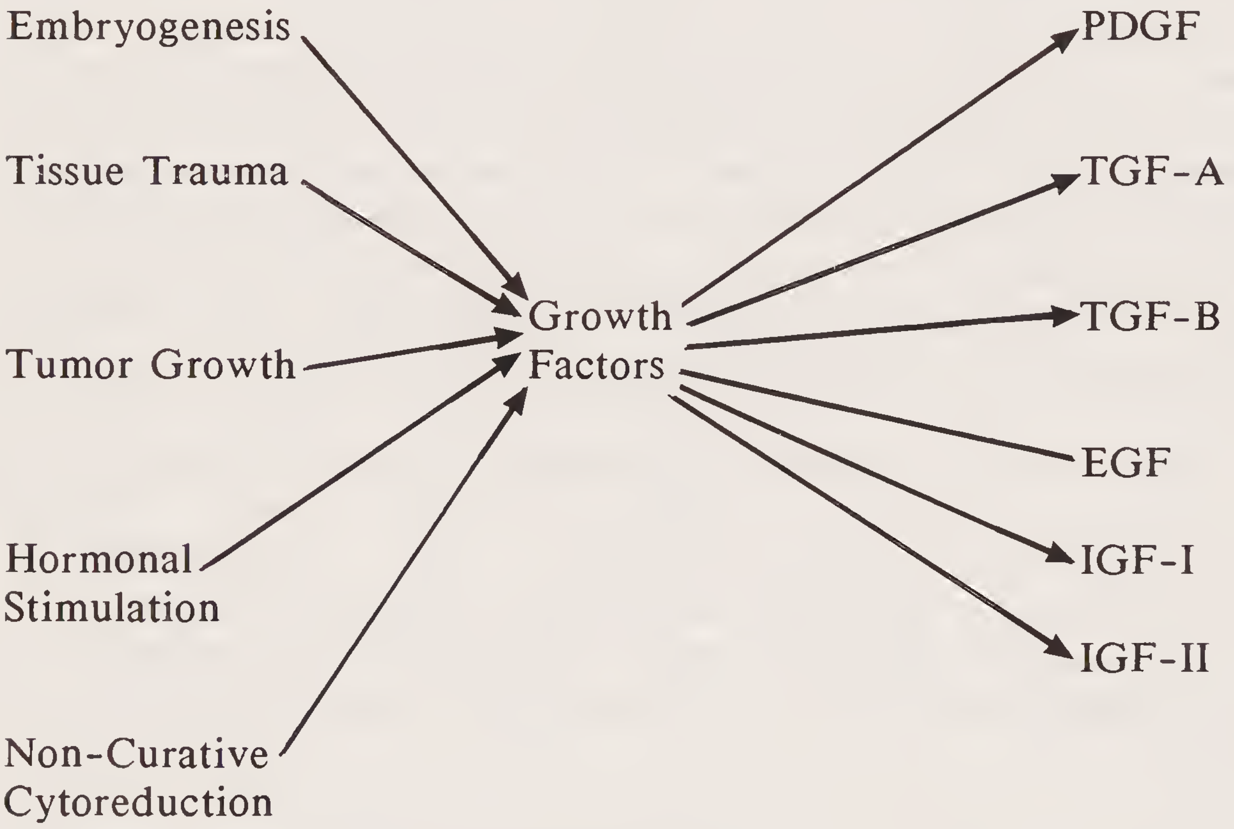
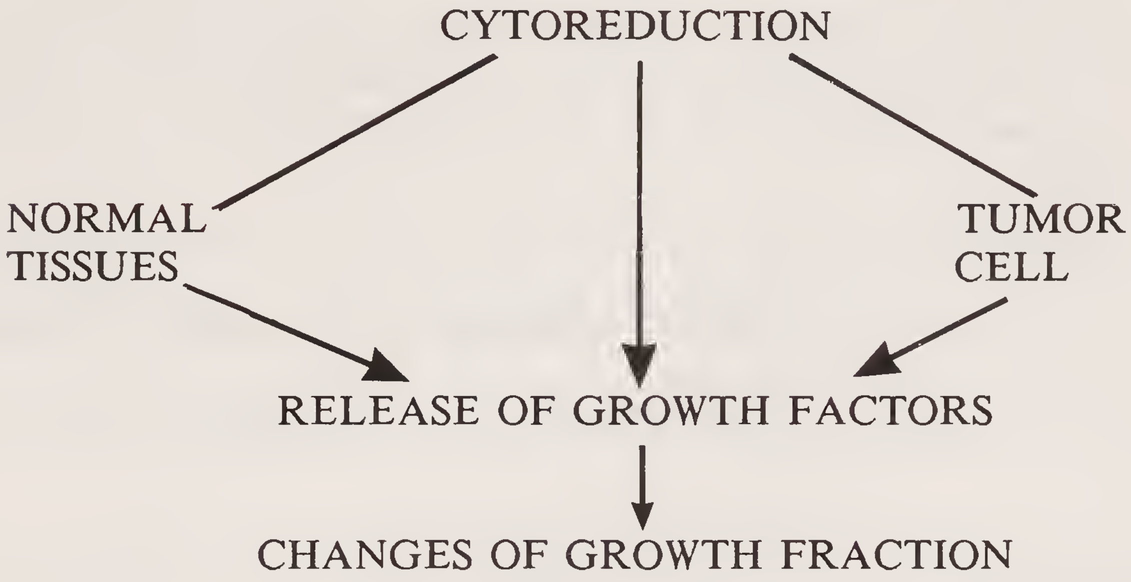


TABLE VIII

EFFECT OF CYTOREDUCTION ON TUMOR GROWTH FRACTION



The association of the presently described growth factors with the malignant process, in either stimulatory or inhibitory fashion, has been documented, and the question of their significance is also being raised in the initiation of the malignant transformation. Their role in mediating the acceleration of the cellular proliferation, after trauma and partial resection under physiological conditions, has been well documented in organs such as liver and kidney (Fidler, 1984; Harris, 1975; Weiss, 1980; Stiles et al, 1985). It seems perfectly reasonable to expect their involvement in tumor growth, particularly as experimental evidence for such an association is already available.

In this context, their generation in tissues of individual organs, and also in fibroblasts, platelets or connective tissue, may not only stimulate the involved organs in an autocrine manner (to repair the locoregional site of the injury), but may also stimulate, as an associated side event, the tumor growth. Such mechanisms are also expected to play a dominant role in mediating alterations of growth fraction after cytoreduction (Table VIII).

TABLE IX

EFFECTS OF HEPATECTOMY AND NEPHRECTOMY ON TUMOR GROWTH

EFFECT OF HEPATECTOMY AND NEPHRECTOMY IN NUDE MICE ON GROWTH OF HUMAN COLON AND RENAL TUMORS (FIDLER, 1989).

	INCREASED GROWTH	**FRACTION OF:**
	COLON CA	RENAL CA
HEPATECTOMY	+++	-
NEPHRECTOMY	-	+++

Of great relevance in this context is work by Fidler who recently summarized his experimental data on growth of tumors in connection with organ repair (Fidler, 1989). Growth factors generated in connection with tissue repair are organ specific, and their stimulatory effects are well documented. In Fidler‘s experiments, the partial hepatectomy, with 75% of the liver removed, stimulated within 4 - 5 days the hepatic, but not renal cells, and within 7 days, 80 - 90% of resected liver was regenerated. When human colon cancer cells were injected into nude mice undergoing liver regeneration, in parallel with the increase of hepatocellular re-growth, a significant stimulation of colon, but not renal malignant cells, was documented (Table IX).

Hence, growth factors released in hepatectomized animals are specific for gastrointestinal but not for uroepithelial cell lineage. In nephrectomized animals, on the other hand, the renal but not hepatic regeneration was documented in surviving animals, and human renal, but not human colon cancer cells were significantly stimulated if injected into nude mice undergoing compensatory nephrectomy. The magnitude of the stimulation after nephrectomy was similar in human renal cancer cells injected subcutaneously, intrarenally, or when compared to established human renal cancer metastases in lungs.

These experiments provide a strong evidence for the role of systemically active growth factors released at the time of the cytoreduction, stimulating both malignant and nonmalignant receptive cells, particularly if associated with tissue specific injury.

GROWTH FACTORS AND TUMOR GROWTH - CONCLUSION

There have been no studies testing the association of cytoreduction and changes of growth fraction in human breast cancer after procedures such as open biopsy, or after noncurative definitive mastectomy. In view of such an association seen in animal studies, it would be prudent to consider a probability for a stimulation of locoregional or systemic micrometastases after these procedures to vary from low to high, but in all likelihood, to be higher than zero.

Hence, precautions such as a reduction of tissue trauma, or modulation of tumor growth by preoperative and postoperative tumor specific therapy, should be explored. Suppression of stimulatory growth factors, or enhancement of inhibitory factors with monoclonal antibodies directed against the growth factors or their receptors, is a realistic possibility (Pollak, 1989). Furthermore, isolation of genes and their products, some of them shown to be growth factor receptors or the growth factor proteins themselves (Fitzpatrick, 1984), offers a major potential for therapeutic intervention via genetic manipulation. Hence, the systemic nature of cell growth communication mediated by stimulatory and inhibitory growth factors seems to be a part of the physiological growth process well preserved in the evolution of not only humans and animals, but also in plants and likely all living species.

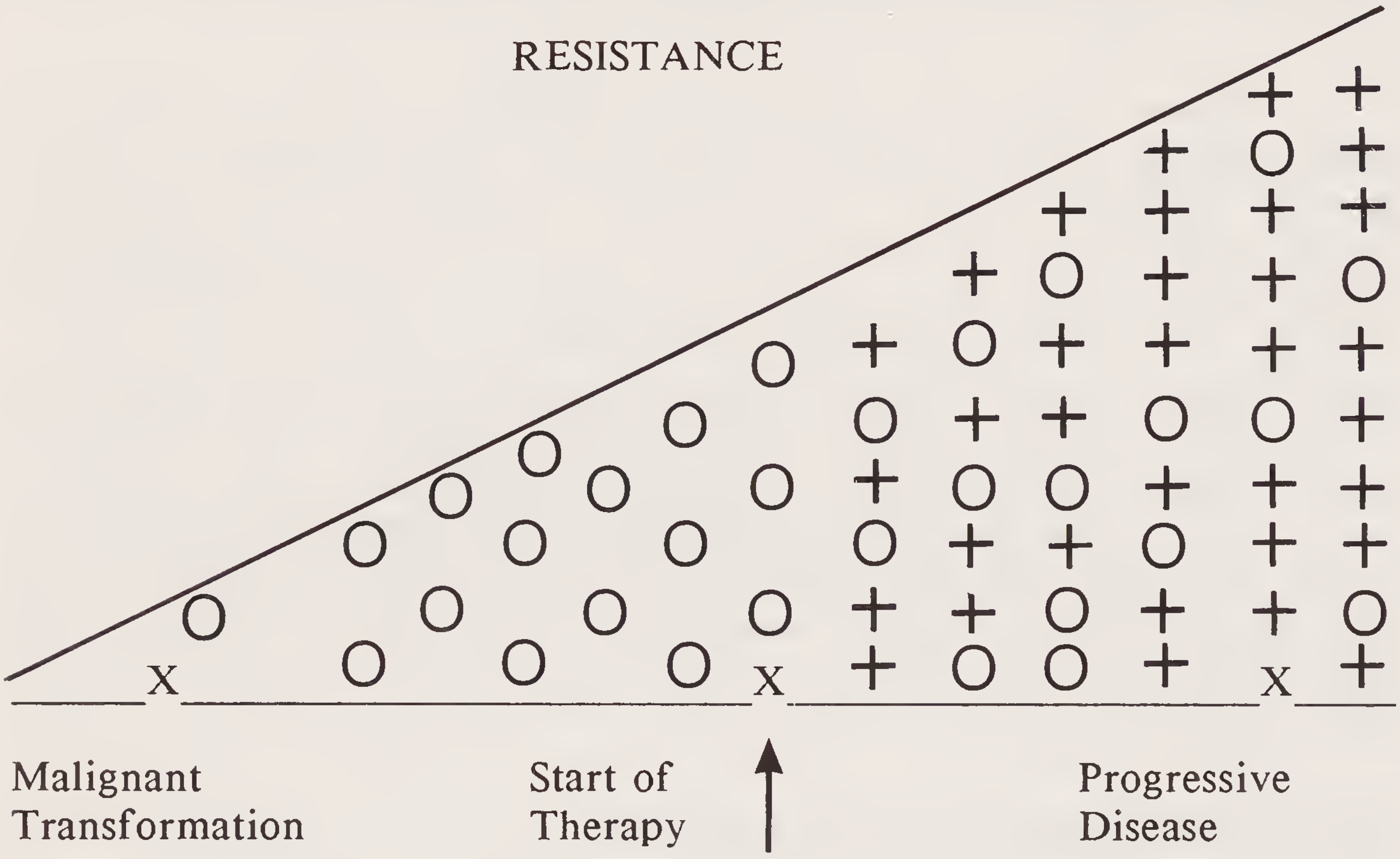
RESISTANCE AND TUMOR KINETICS

Is there any possibility that a more rapid cell division rate as induced by the surgery in the postoperative time period will increase the chance of mutational changes leading towards resistance? Will even a minor reduction of the time delay of instituting systemic chemotherapy have an impact on reducing the resistance?

Tumor resistance at the cellular level is considered to be a genetic phenomenon. Genes code for enzymes and for the overall complex proteins subsequently responsible for functional phenomena such as drug metabolism, injury repair, or transmembrane kinetics of therapeutic agents. The somatic mutation theory of Goldie and Coldman (Goldie and Coldman, 1979), Ling's more recent work on the p-glycoprotein effusion pump (Kartner and Ling, 1989), as well as the growing number of studies confirming the presence of the multidrug resistance genes, all attest to the importance of cellular genetic events in the formation of resistance. However, the most provoking question is whether these genetic events are present from the early time of tumor transformation, and hence before the

TABLE X

CONTRIBUTION OF "THERAPY INDEPENDENT" VS "THERAPY INDUCED" RESISTANCE TOWARDS TOTAL RESISTANCE



Legend:

Resistance as a Result of Therapy: +

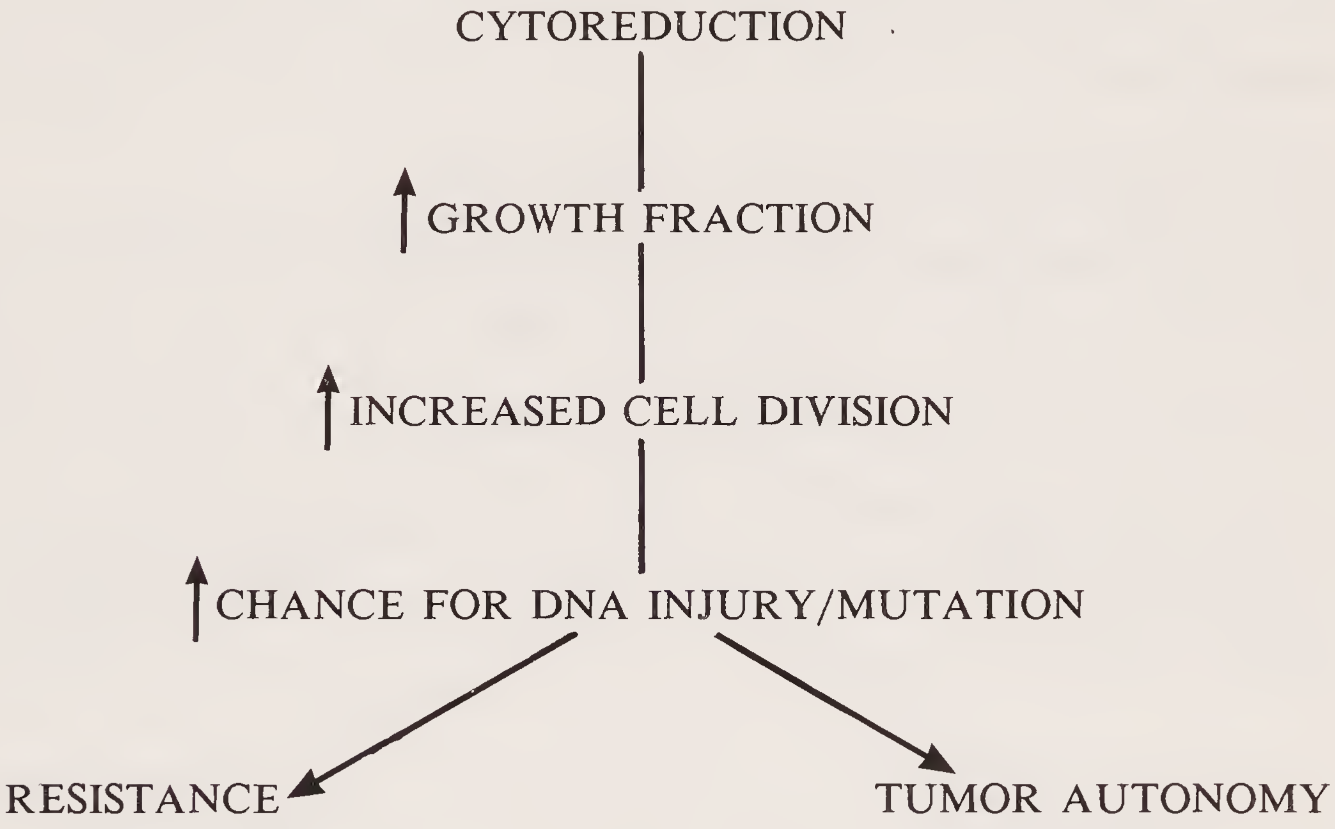
Resistance Independent on Therapy: O

diagnosis, as part of the inherent tumor characteristic, as opposed to their development during the process of established disease. Both mechanisms are likely present in a given tumor, albeit in a different proportion (Table X).

The inherent resistance, representing the tumor intrinsic genetic events, is beyond the reach of therapeutic manipulation, as resistant cells are present before the earliest treatment can be applied. If, however, the resistance is an acquired phenomenon developing after diagnosis, then the manipulation of the timing of initiating therapy may have very significant implications toward curability. Furthermore, it is during the time of cellular division that the genes are activated, and during the S-phase that the DNA is most vulnerable to mutational injuries leading towards the resistance. It is, therefore, very plausible that the postcytoreductive increase in the rate of cell division may be a critical time period leading to an increase of resistant mutants (Table XI).

TABLE XI

ASSOCIATION OF CYTOREDUCTION AND RESISTANCE

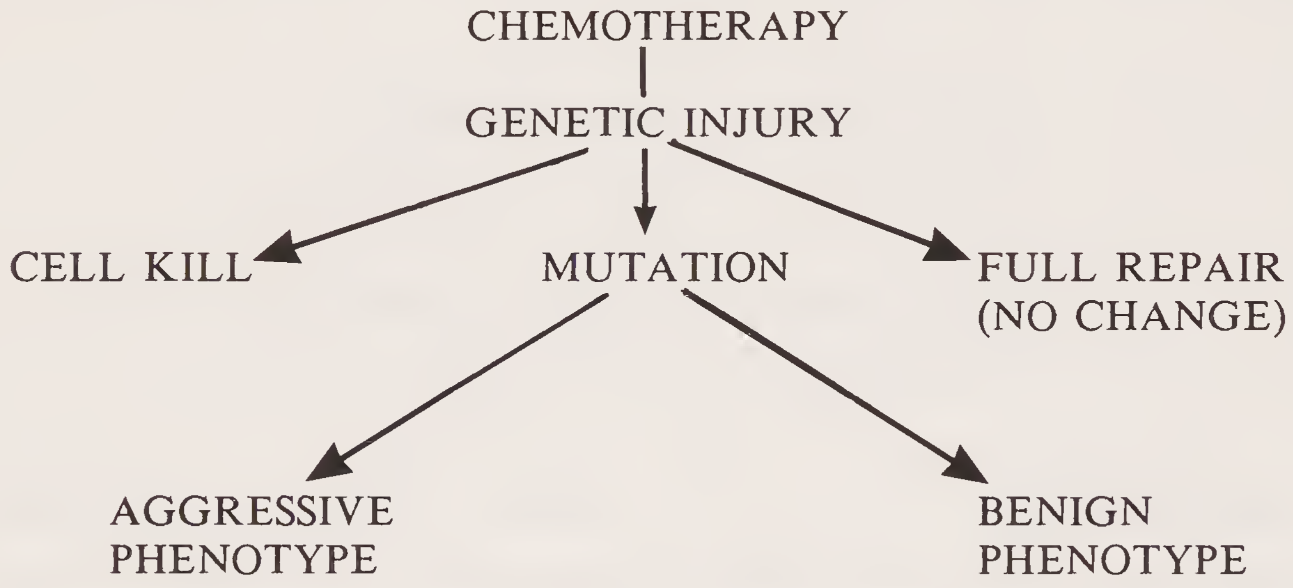


EFFECTS OF PREOPERATIVE CHEMOTHERAPY ON THE METASTATIC POTENTIAL OF SURVIVING TUMOR STEM CELLS

The preoperative timing of chemotherapy may, in the context of the above-mentioned arguments, render benefit for two reasons. Firstly, the reduction of the time between diagnosis and therapy may be the most critical factor in those tumors in which the first resistant mutant arises during this time delay. Such development would apply particularly for tumors with a low doubling time and a high mutation rate. The second expected benefit of preoperative chemotherapy involves the biology of cellular behavior, as expressed by many workers in studies on differentiation (Lotem and Sachs, 1980; Rocchi et al., 1987; Schwartz and Sartorelli, 1982; Thiele et al., 1985).

TABLE XII

CHEMOTHERAPY EFFECT ON TUMOR DNA



As a result of the cytotoxic effect of chemotherapy, there will be, at the time of surgery, an overall reduction of the tumor stem cell burden at risk for postcytoreductive kinetic acceleration. It has been shown that tumor stem cells exposed to, but not killed by chemotherapy, may leave the dividing cell compartment, and that chemotherapy pre-treatment may abrogate the increase of labelling index documented in animals undergoing noncurative surgery without preoperative therapy (Fisher, 1989). If the increased capacity of stem cells to divide postoperatively is truly reduced by preoperative chemotherapy, then also their motility, the transgression through the capillary wall and their survival in the hostile environment of the circulation and distant organs, may all be greatly diminished (table XII).

Although the most fundamental action of chemotherapy is a direct cell kill, a partial damage without immediate cell death is yet another feature of the chemotherapy or radiation effect. Such surviving, but damaged cells may appear indistinguishable from invasive malignant cells under the light microscope, but as a population of "doomed" cells, may undergo several divisions and subsequently move from the cycling into the noncycling compartment. Also, many agents, including retinoic acid or other more commonly used chemotherapeutic and hormonal agents, have shown their capability of inducing terminal differentiation, reducing in this way, and in various degree, the metastatic potential of the tumor (Lotem and Sachs, 1980; Rocchi et al., 1987; Schwartz and Sartorelli, 1982; Thiele et al., 1985; Biedler et al., 1983; Thiele et al., 1988; Biedler et al., 1988).

Studies on neuroblastoma (Rocchi et al., 1987; Thiele et al., 1985; Thiele et al., 1988), and HL-60 leukemia (Lotem and Sachs, 1980; Schwartz and Sartorelli, 1982) have shown that several commonly available differentiating and chemotherapeutic agents are able to induce differentiation of anaplastic cells into a more benign phenotype. Whether effective preoperative therapy of solid tumors will achieve similar results remains to be shown. If true, such altered tumors, although histologically indistinguishable from invasive tumors not exposed to therapy, may acquire different metastatic characteristics, resulting in either a more aggressive, or alternatively, a more benign malignancy (Table XII).

CONCLUSION

In this chapter we have discussed some evidence, mainly from experimental studies, implicating the cytoreduction and tissue injury with increased kinetical and other biological alterations of the preformed or newly formed metastases in the early postsurgical period. While interaction with growth factors and other refined mechanisms regulating tumor cell growth would be the most specific treatment to abrogate these potentially deleterious changes, presently, more easily constructed steps like the reduction of the extent of radical surgeries,utilization of needle instead of open biopsies, and testing approaches like preoperative therapy, and or utilization of regimens of maximally tolerated dose intensity, are all examples of treatment strategies to be tested against conventional approaches.

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INTRAPERITONEAL CHEMOTHERAPY FOR PERITONEAL CARCINOMATOSIS FROM COLONIC OR APPENDICEAL CYSTADENOCARCINOMA: RATIONALE AND RESULTS OF TREATMENT.

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INTRODUCTION

It is well known that the biological aggressiveness of the malignant process varies greatly from primary site to primary site and from patient to patient. Some primary tumors, even though minute in size, will result in a wide spread metastatic process. Other tumors may grow locally to extraordinary size and never result in distant metastases. Not only does the tendency of a tumor to spread via lymphatic channels or via the blood stream vary greatly. Its tendency to invade locally may result in deep penetration into the surrounding tissues or merely result in a superficial proliferation of malignant tissue. In colon cancer the deeply penetrating lesions are usually characterized by an ulcerative primary cancer; the more superficially invasive cancers are often polypoid in their gross appearance. Seemingly unrelated to a primary tumor's ability to metastasize or its ability to invade locally is a cancer's ability to implant on a raw tissue surface. The cancer discussed extensively in this manuscript is the grade 1 cystadenocarcinoma. This cancer does not metastasize through the blood stream or via lymphatic channels. This cancer does not invade locally into adjacent organs or structures. Yet, this tumor will implant on virtually every peritoneal surface if tumor cells are exfoliated into the abdominal cavity. The pharmacologic rationale for chemotherapy for cystadenocarcinoma of the colon or appendix is high regional drug concentrations for extended time periods. A review of the results of treatment including gross and histologic changes induced by intraperitoneal chemotherapy shows responses in most patients. Peritoneal carcinomatosis from grade I cystadenocarcinoma of the perforated colon or appendix is no longer of necessity a fatal condition.

NATURAL HISTORY OF COLONIC ADENOCARCINOMA AND GRADE I CYSTADENOCARCINOMA COMPARED

One of the important contributions to our understanding of gastrointestinal malignancy comes from "Failure Analysis". Careful follow-up of patients after surgical treatment of gastrointestinal malignancy has resulted in an accurate tabulation of the anatomic sites of surgical treatment failure. Cunliffe and Sugarbaker have carefully itemized the sites of large bowel cancer recurrence in these 50 percent of patients who develop recurrent disease despite the surgeons ability to achieve a "potentially curative operative procedure"(1). Figure 1 schematically presents the anatomic sites at which recurrent malignancy has been recorded with colonic adenocarcinoma reoperative series and at autopsy. The most common site for colorectal cancer to recur is the resection site. Although not recognized by most surgeons, local recurrence in the portion of the abdomen or pelvis from which the surgeon removed the primary tumor is the most common anatomic site for its recurrence. A second prominent site of surgical treatment failure is peritoneal surfaces. Liver metastases occur in approximately 50 percent of patients at the time of autopsy. At the time of first recurrence they are seen in only 30 percent of patients.

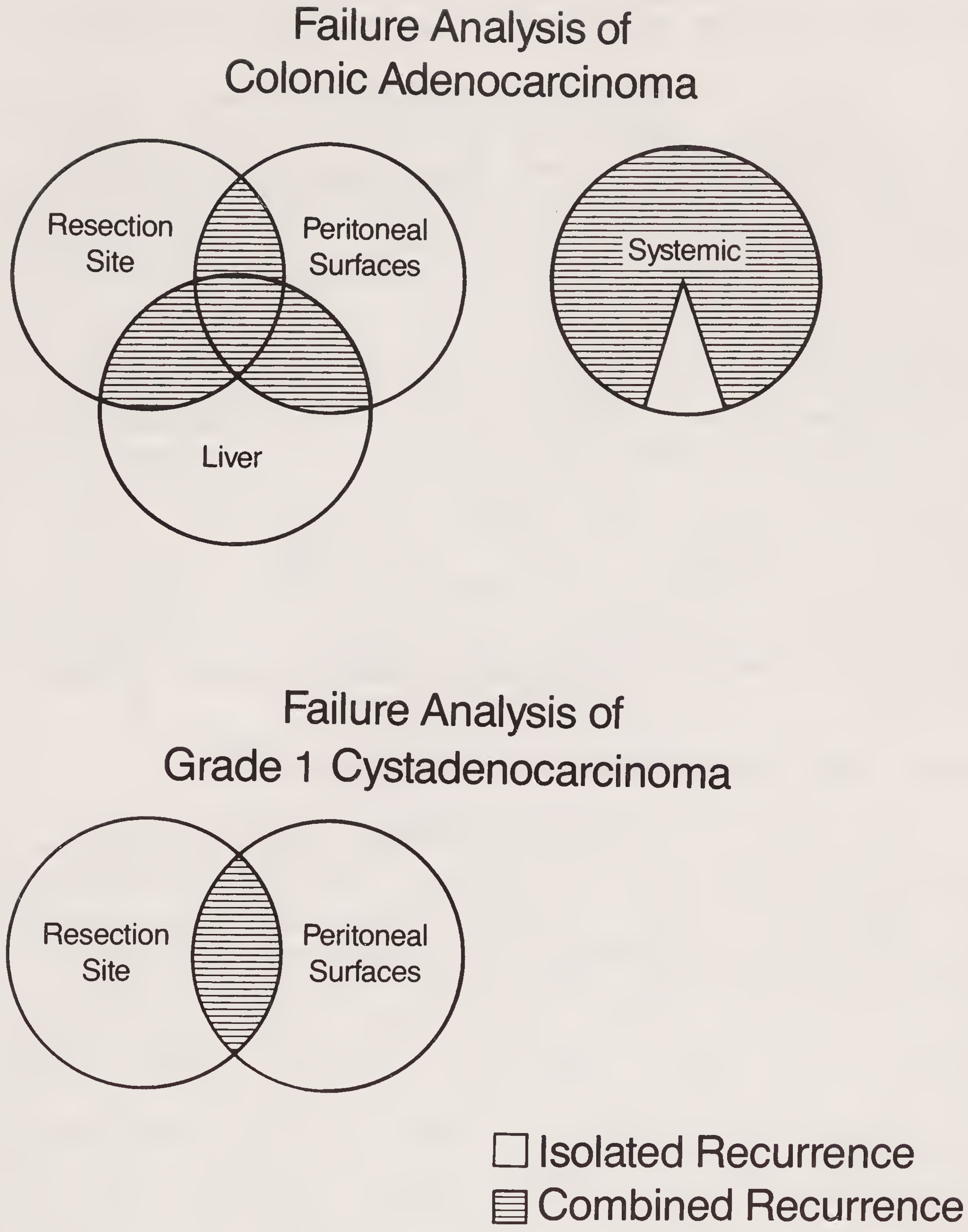


Figure 1. Recurrence patterns of colonic adenocarcinoma and grade 1 cystadenocarcinoma compared.

Systemic metastases are also seen but are unusual as the first site of recurrent cancer.

Figure 1 contrasts the recurrence patterns of colonic adenocarcinoma and grade 1 cystadenocarcinoma of the appendix or colon. Grade 1 cystadenocarcinoma comprises approximately 75 percent of appendiceal malignancies but only approximately 5 percent of colon or rectal tumors. It often presents as a progressive abdominal swelling from mucinous ascites (2). If this primary tumor is excised its recurrence patterns are markedly different than colonic adenocarcinoma. The cancer does not metastasize hematogenously to the liver, via lymphatics to the lymph nodes, nor does it deeply invade into adjacent organs or structures. However, the tumor has a relentless recurrence pattern at the resection site and on the peritoneal surfaces throughout the abdominal cavity.

TUMOR CELL ENTRAPMENT HYPOTHESIS

How does one explain the high incidence of resection site and peritoneal surface recurrence in patients with gastrointestinal malignancy? At the completion of the surgical procedure no tumor can be seen at either of these sites. It is unlikely that resection site recurrence represents tumor regrowth within lymph nodes. It is the surgeons task to remove all the regional lymph nodes so that a generous portion of normal tissue separates the tumor from the margin of resection. When Lofgren and co-workers examined local colorectal cancer recurrence, they did not find disease to be present within lymph nodes (3). Sugarbaker and colleagues have suggested that the high incidence of local-regional cancer recurrence with gastrointestinal malignancy can be explained in terms of the Tumor Cell Entrapment Hypothesis (4). The theory is shown schematically in Figure 2. This hypothesis suggests that tumor cells dislodged from the lateral margins of resection, released from

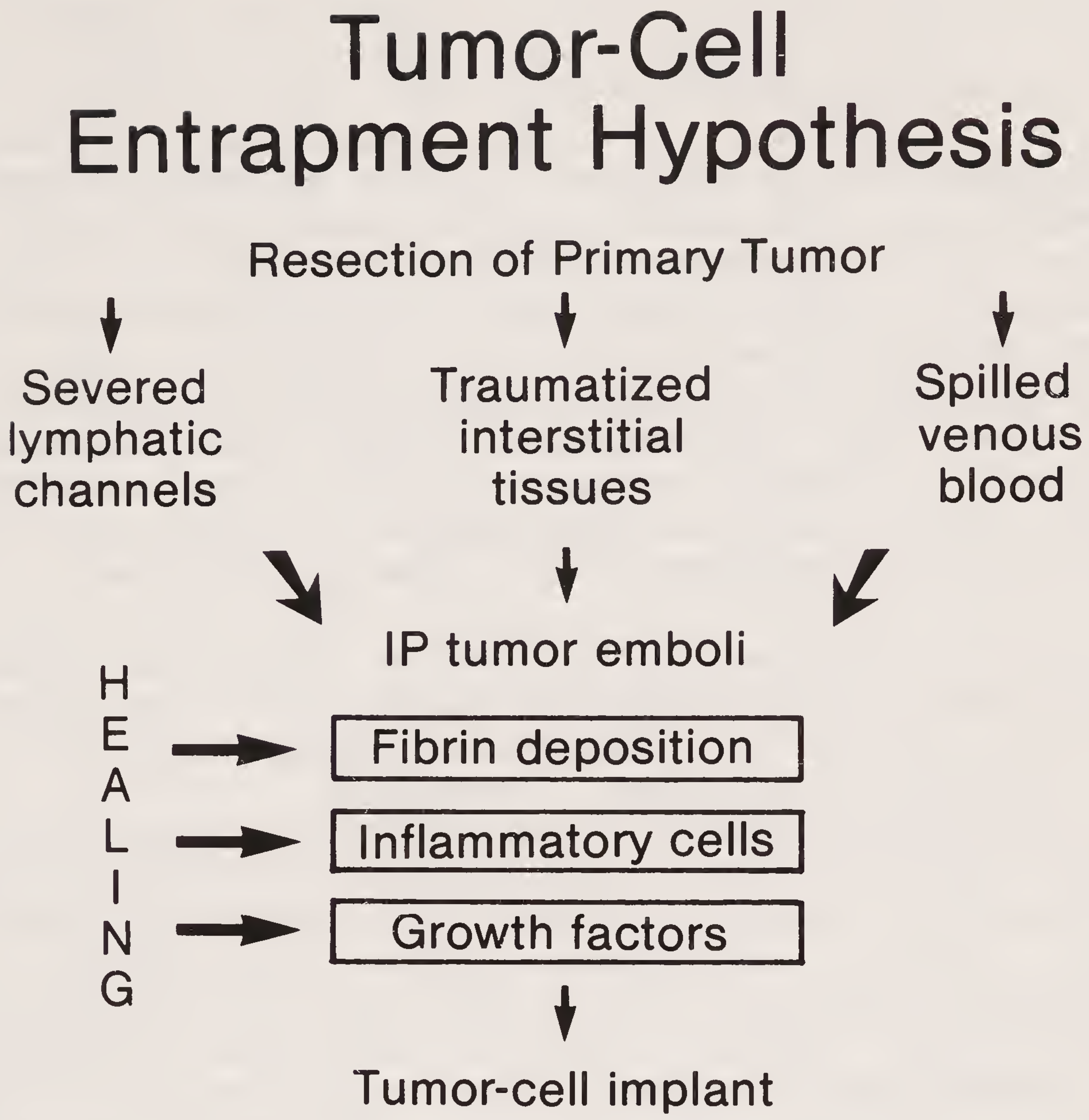

Tumor-Cell Entrapment Hypothesis
Resection of Primary Tumor
Severed lymphatic channels
Traumatized interstitial tissues
Spilled venous blood
IP tumor emboli
HEALING
Fibrin deposition
Inflammatory cells
Growth factors
Tumor-cell implant

Figure 2. Tumor cell entrapment hypothesis.

lymphatics by cutting through lymphatic channels, and spilled along with the venous blood into the abdominal cavity are responsible for intraperitoneal tumor emboli. These tumor cells are trapped within the abdominal cavity after closure of the abdominal incision. Malignant cells adhere to raw tissues surfaces within the abdominal cavity and are fixed in place by a layer of fibrin. The most prominent sites that trap tumor emboli are the resection site and abraded peritoneal surfaces. It is a common observation at the time of a reoperative surgical procedure to find tumor implants located on bowel loops close to the resection site and likely to be traumatized by surgery, but not on small bowel at other locations.

Local-regional treatment failures become increasingly important as a tumor's tendency to metastasize or to invade decreases. With grade 1 cystadenocarcinoma treatment failure will exclusively result from a few tumor cells exfoliated from the appendiceal or colonic tumor mass prior to its resection. Regional agents that exert antitumor effects within the abdominal cavity after removal of the primary tumor or after removal of more extensive intraabdominal spread may lead to prolonged disease free survival in this patient population.

THEORY OF METASTATIC INEFFICIENCY

Weiss and colleagues have presented data to show that large numbers of tumor cells within endothelial lines spaces may be well tolerated by the host (4). Indeed even 10^7 cells may be destroyed without any liver metastases resulting following intraportal tumor injection. The lungs also show considerable resistance to tumor cell implantation when these cells reach the lungs via the blood stream. However, tumor cells that implant on a raw tissue surface and are then buried by a fibrinous exudate present a special problem. The efficiency for tumor cell implantation in this situation is greatly increased. For the surgeon, the tumor cell entrapment hypothesis means that even a few tumor cells lost into the free peritoneal cavity as a result of the surgical dissection may result in surgical treatment failure. Large numbers of tumor cells confined to the vascular and lymphatic spaces may not result in recurrent cancer. For grade 1 cystadenocarcinoma the tumor cell entrapment hypothesis has profound meaning. This tumor does not metastasize hematogenously or lymphatically and this tumor does not extensively invade. However it is able to implant extensively within the abdominal cavity. Surgical experience would suggest that this tumor tends to recur most densely within the anatomic site from which the primary tumor was removed and then within small and large bowel adhesions that are created close to the resection site by intraoperative trauma.

Figure 3 shows the routes for dissemination of colorectal malignancy. The tumor cell entrapment hypothesis is of surgical relevance to colonic adenocarcinoma in that there is a relatively greater likelihood of tumor cells implanting on raw surfaces than of implanting within endothelial lined vascular or lymphatic channels. However, if there is systemic spread then the clinical significance of intraabdominal tumor implantation becomes diminished.

For grade 1 cystadenocarcinoma the metastatic inefficiency via blood or lymphatics approaches infinity. This means that this tumor almost never metastasizes by these routes. However, the metastatic inefficiency of local spread by implantation is extremely low. This implantation rate is, presumably, at least as high as the implantation rate from the more common colonic adenocarcinoma. However, because it is the only route of disease dissemination it becomes much more important to the care of the patient to prevent this sole route of dissemination of tumor emboli.

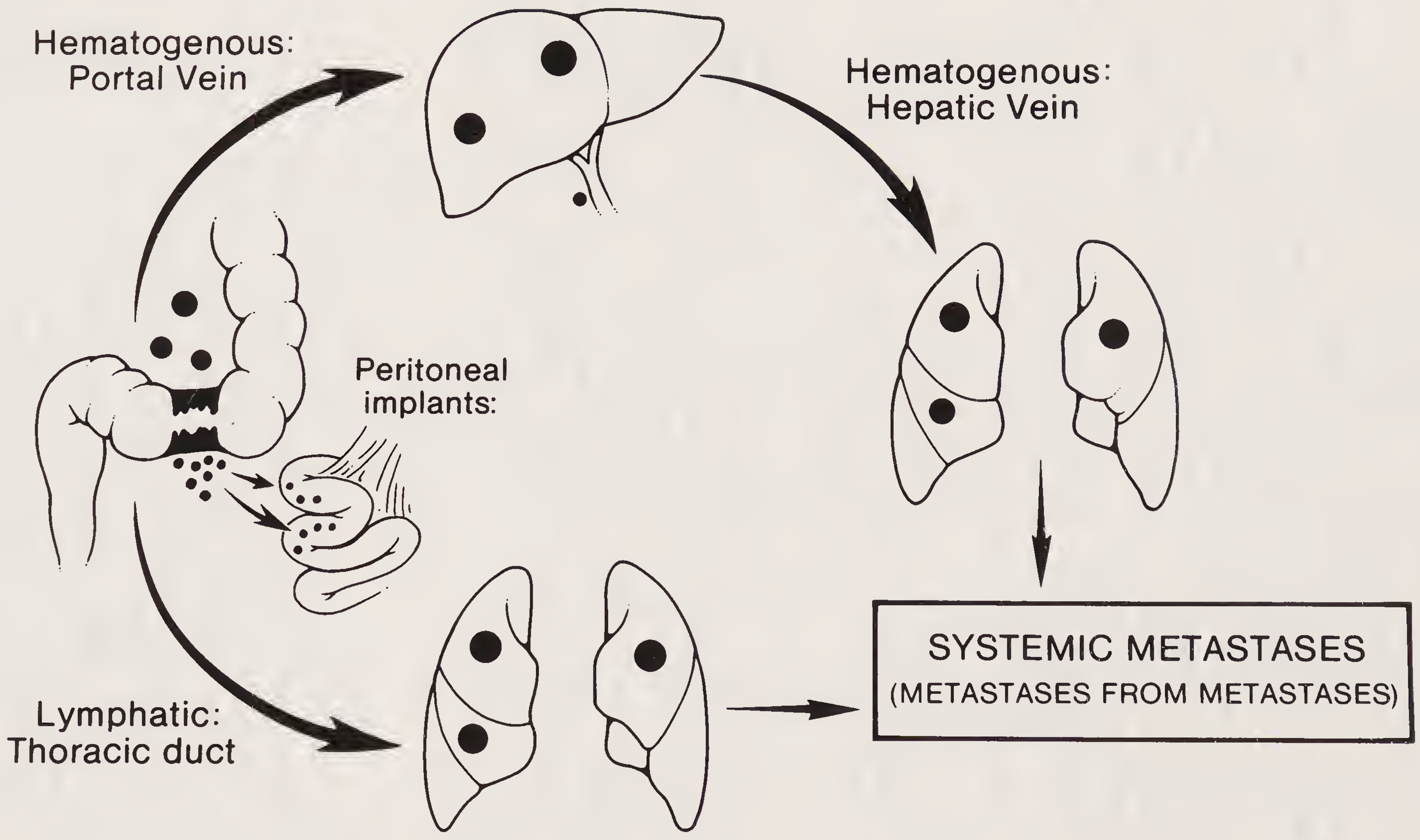


Figure 3. Routes of spread of colonic adenocarcinoma.

REQUIREMENTS FOR ADJUVANT TREATMENT STRATEGIES

Colonic adenocarcinoma shows an implantation efficiency that is much greater when tumor cells are disseminated onto raw tissue surfaces as compared to the hematogenous or lymphatic routes of dissemination. However, because more than one route of tumor dissemination is important, the regional adjuvant cancer therapies must be inadequate. Only if all of the sites of treatment failure can be regionally treated, does an intraperitoneal approach to adjuvant systemic therapy become reasonable. For optimal adjuvant therapy for colonic adenocarcinoma one must treat the resection site, peritoneal surfaces, the liver, and anatomic sites for systemic disease dissemination including the lungs, adrenal glands, and even the brain.

For colonic or appendiceal cystadenocarcinoma a completely adequate adjuvant surgical treatment strategy could have a definite impact on survival and only exert regional effects. We will try to show in later paragraphs that intraperitoneal chemotherapy can, from a theoretical point of view, be the optimal adjuvant therapy for grade 1 cystadenocarcinoma that can be surgically removed with minimal residual disease.

UNIQUE CLINICAL FEATURES OF GRADE I CYSTADENOCARCINOMA FROM COLON OR APPENDICEAL CARCINOMA

As reviewed in previous paragraphs grade I mucinous adenocarcinoma has a unique natural history. This results in an unusual clinical history and, potentially gives the tumor unique treatment opportunities. The unique features of grade 1 cystadenocarcinoma of the large bowel are itemized in Table 1. Because the tumor is minimally invasive and creates virtually no scirrhous features it rarely if ever causes bowel obstruction or bleeding. In the bowel lumen the villous adenoma may be the clinical counterpart of mucinous cystadenocarcinoma. The most common presentation of this malignancy is a gradually distending abdomen that results from copious mucinous ascites (2). The surgeons finds, upon abdominal exploration, widespread tumor deposits on all abdominal surfaces, especially the omentum. Figure 4 shows the "omental cake" that is the hallmark of malignant pseudomyxoma peritonei. However, tumor is also characteristically lodged in all of the dependent portions of the abdomen. Prominent sites for massive tumor accumulation include the left upper quadrant surrounding the spleen, the lesser omentum including portahepatis, both right and left abdominal gutters, the right subhepatic and right suprahepatic spaces, the pelvis, and the left iliac fossa just lateral to the sigmoid colon. At the initial surgical procedure the small bowel is almost always relatively spared of the malignant process. Perhaps the continuous motion of the small bowel tends to wipe off tumor implants over the months and years that this process takes to develop.

Most patients dying of this disease suffer with a confluence of tumor within the abdominal cavity and an unrelenting increase in intraabdominal pressure from the mucinous ascites. Even at autopsy hematogenous and lymphatic disease dissemination is not seen. This unique feature of this low grade intraabdominal malignancy makes intraperitoneal chemotherapy an adequate, perhaps even ideal, adjuvant surgical treatment strategy.

Not only is this a non-metastasizing tumor but it is also a non-invasive malignancy. The primary tumor does not cause a scirrhous reaction so that the bowel lumen is not narrowed from either the primary tumor or from implants on the bowel surface. This non-invasive character allows one to apply a surface agent that will destroy tumor to some depths, then come back some time later and reapply this agent and expect sequential tumor destruction. This

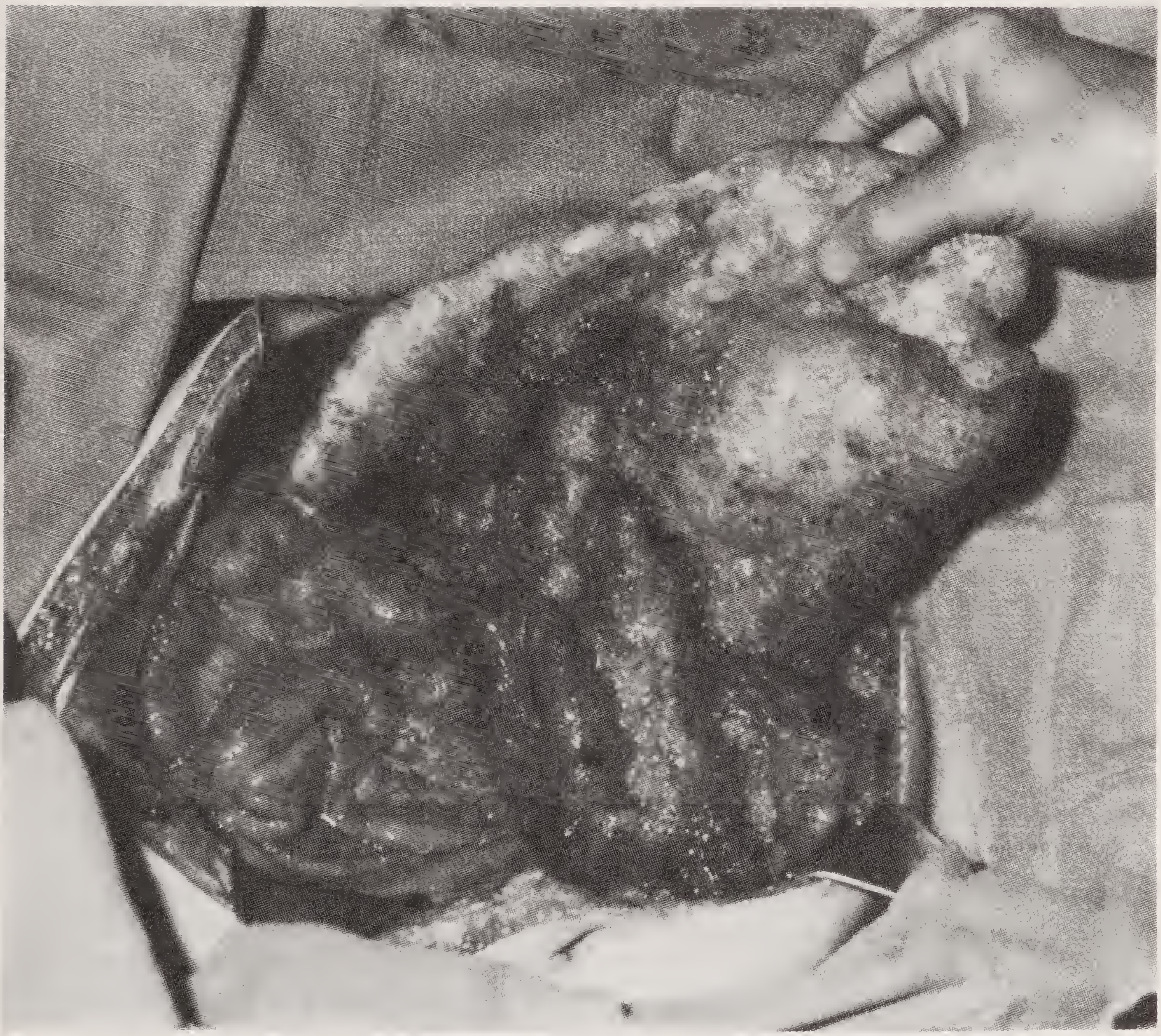


Figure 4. The hallmark of pseudomyxoma peritonei is the "omental cake". The patient shown in this figure is a six year survivor free of disease.

means that multiple cycles of intraperitoneal chemotherapy over the course of time may be expected to remove the tumor layer by layer. As long as the chemotherapy comes in contact with the tumor, and one is careful not to irreparably damage other structures (such as the visceral surface of the small and large bowel and pancreas parenchyma) these treatments can be continued until all cancer has been destroyed layer by layer.

Most cancers grow as a spherical mass until they reach a certain size and then disseminate the malignant process to other anatomic sites. The spherical tumor mass is poorly supplied by blood capillaries and lymphatic channels. The partially avascular tumor is generally poorly penetrated by chemotherapeutic agents and this is one reason for the low proportion of responders even with the doses used in intensive treatment regimens. Cystadenocarcinoma presents a far different physical situation for the delivery of chemotherapy. Tumor cells are suspended in a mucin matrix. This mucinous material is water soluble. Chemotherapy put into the abdominal cavity in a large volume of fluid will diffuse into this water soluble matrix along a diffuse gradient. Chemotherapy will also diffuse into normal structures; however, because of the rich capillary and lymphatic channels, the drugs will rapidly be removed from these sites. In contrast, the mucinous tumor has a poorly developed lymphatic and capillary network. Neither does it have the enzyme systems by which to metabolize the chemotherapeutic agents. Therefore cytotoxic doses of drug that diffuse into the mucinous matrix that surrounds these tumor cells will persists for a long time period. This leads to destruction of a larger proportion of tumor cells with cystadenocarcinoma that with solid tumor nodules. Also, it accounts for the fact that normal structures, for the most part, protect themselves from the toxic effects of intraperitoneal chemotherapy. To summarize, intravenous chemotherapy must reach malignant tissue through vascular channels and necrotic tumor tends to go untreated. For intraperitoneal chemotherapy diffusion into tumor does not depend on tumor vascularity. Avascular tumor will tend to retain chemotherapy because there is little lymphatic or capillary supply to remove active drug.

Presumably because of the continuous motion of the small bowel, this portion of the anatomy is relatively preserved from tumor implantation. This is only true upon presentation. After surgical operative procedures the mucinous tumor may become confluent on small bowel surfaces. The tumor recurs within the fibrous adhesions that are caused by abrasions of the small bowel surface secondary to surgery. When tumor recurs within these fibrous adhesions the small bowel may become extensively involved. Figure 5 shows the histologic appearance of pseudomyxoma peritonei before and after surgical efforts to remove this cancer.

Cytoreductive surgery involves an omentectomy - splenectomy procedure. Deperitonealization at other anatomic sites will result in the removal of mucinous tumor adherent to peritoneal surfaces. Because of the noninvasive tumor growth and the tendency to spare small bowel, this surgery can make patients relatively disease free. Seldom is the resection of small bowel or other intraabdominal structures required except as a part of the primary tumor mass. An exception to this is the abdominal colon which sometimes must be removed from ileocecal valve to peritoneal reflection on order to resect omentum and spleen.

PERITONEAL-PLASMA BARRIER

Surprisingly, there is a barrier between the peritoneal cavity and the plasma that is similar

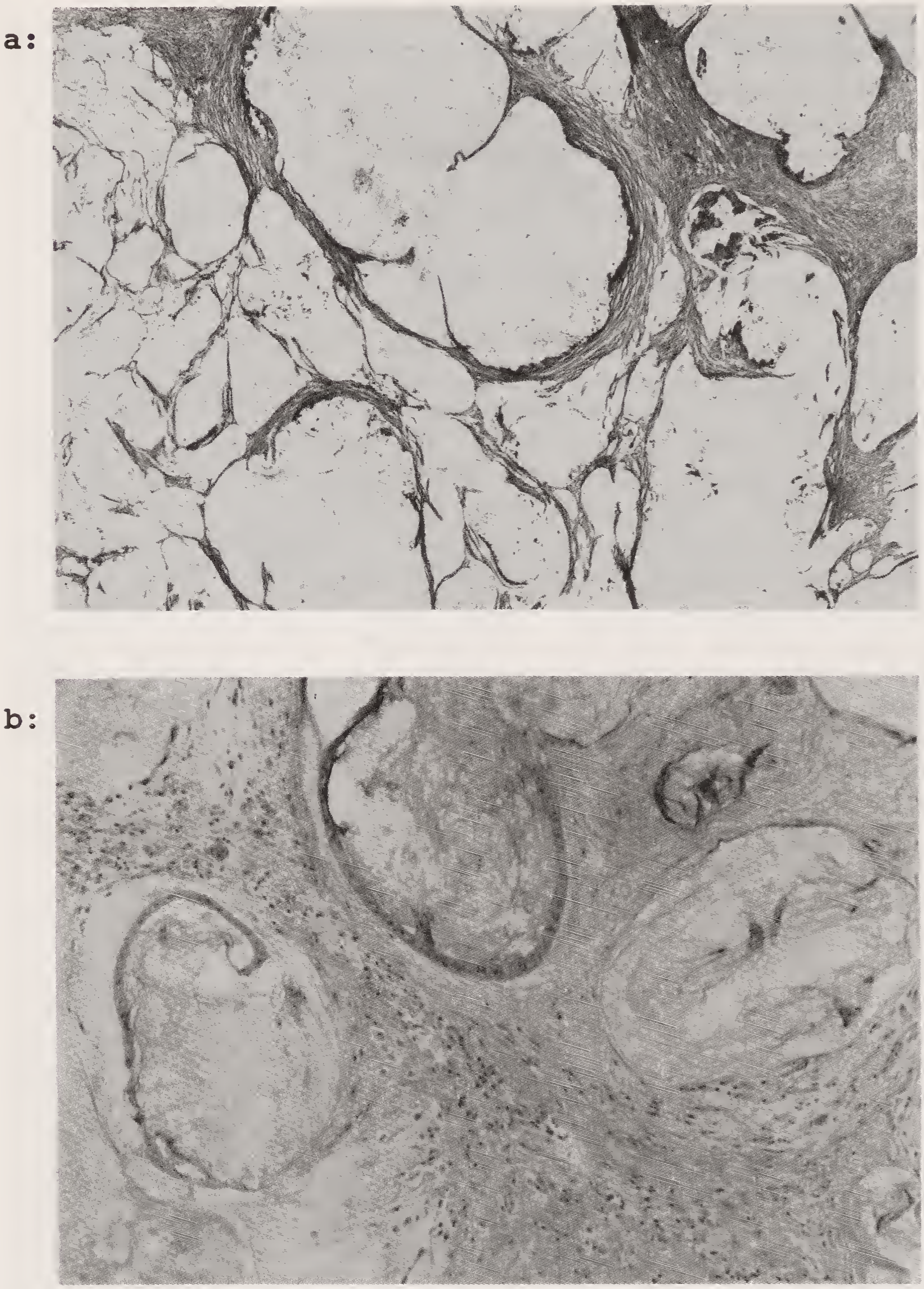


FIGURE 5a: Histologic appearance of pseudomyxoma peritonei. **FIGURE 5b:** Histologic appearance after surgical procedures to remove mucinous tumor. The cancer tends to recur within the scar that constitutes abdominal adhesions.

to the blood-brain barrier. Drugs of large molecular weight that are placed in the peritoneal cavity will be cleared from this anatomic site at a much slower rate than the same substance would be cleared from the vascular compartment. Figure 6 shows the pharmacokinetics of intravenous or intraperitoneal drug delivery of large molecules. These data provided by Flessner and colleagues show that the area under the curve (drug concentrations over time) for intraperitoneal drug delivery may be several logs higher in the abdominal cavity than drug exposure that develops systemically (6). Myers and colleagues have determined that the concentration difference between peritoneal fluid and plasma is, for the most part, dependent on the molecular weight of the substances (7).

PHARMACOKINETICS OF CHEMOTHERAPY INSTILLED IP IN A LARGE VOLUME OF FLUID

In designing novel treatment strategies for cystadenocarcinoma one may theorize that a much greater exposure of the peritoneal cavity to drug effects may be gained by intraperitoneal chemotherapy administration as compared to the systemic route. 5-Fluorouracil (5-FU) is a large molecule that is active against a wide variety of large bowel tumors. Figure 7 shows the pharmacokinetics of intraperitoneal 5-FU administration. Another drug that synergizes 5-FU and that is frequently used clinically along with 5-FU is mitomycin-C (MMC). MMC shows similar concentration advantages in the abdominal cavity after IP drug delivery (8).

RATIONALE FOR EARLY POSTOPERATIVE INTRAPERITONEAL CHEMOTHERAPY (EPIC)

As discussed earlier, tumor cells may become entrapped within the fibrinous material that is deposited widely throughout the abdominal cavity as the body begins the healing process. Tumor

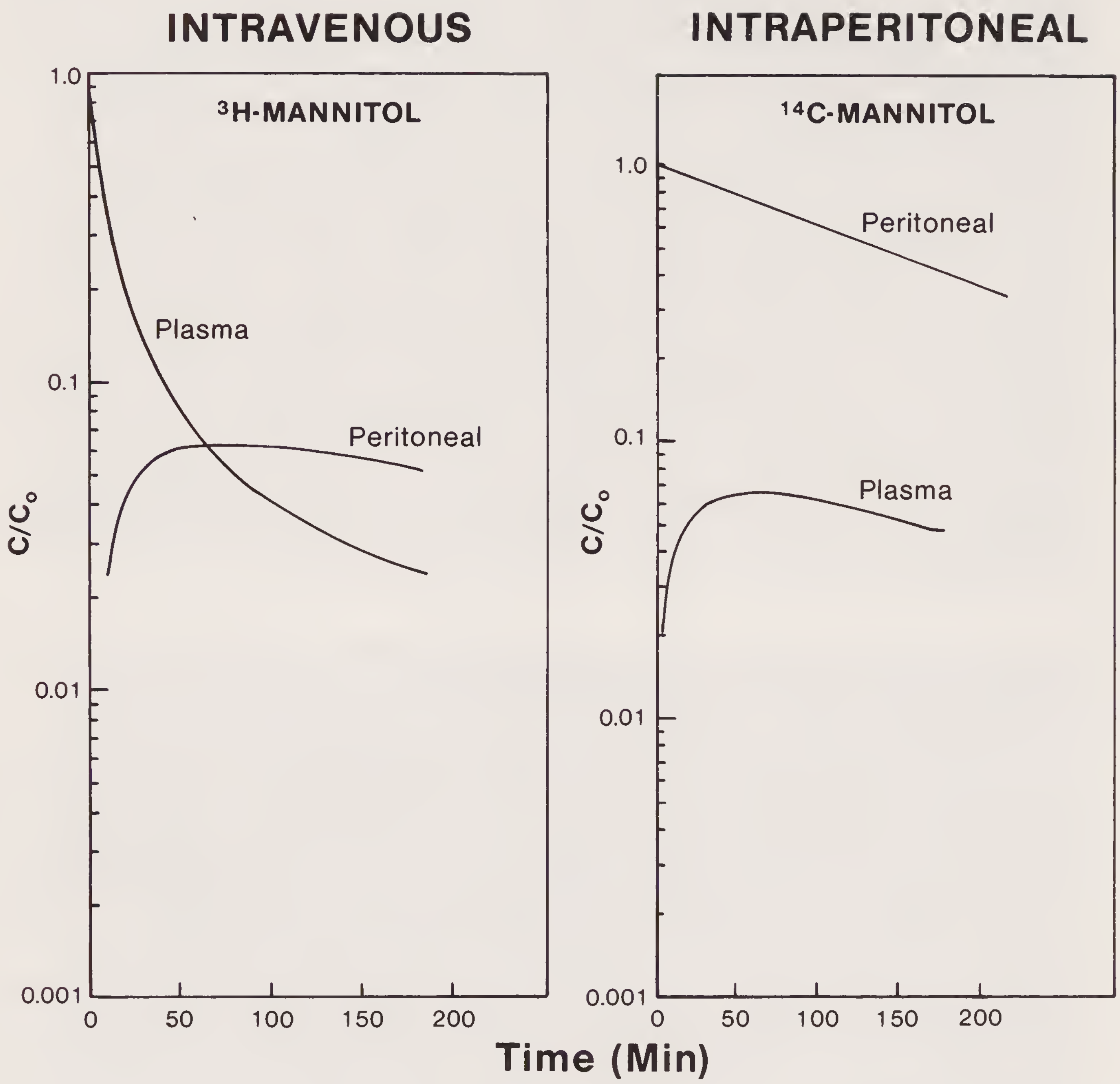
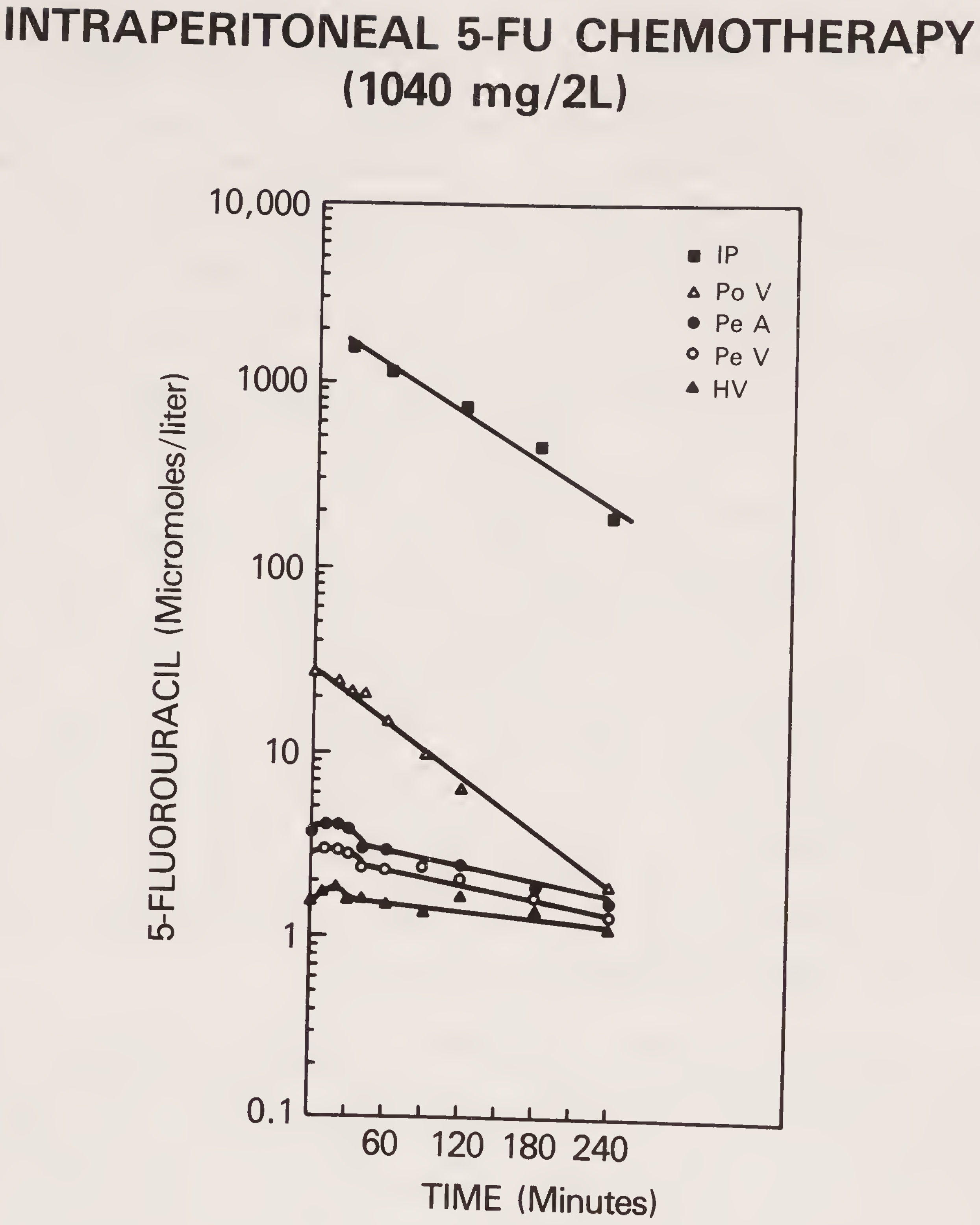


Figure 6. Pharmacokinetics of intravenous or intraperitoneal drug deliver of large molecules (modified from Flessner et al, Reference 6.).



INTRAPERITONEAL 5-FU CHEMOTHERAPY (1040 mg/2L)

Figure 7. Pharmacokinetics of intraperitoneal 5-fluorouracil in a patient with normal peritoneal surfaces.

cells captured in this fibrinous material are expected to implant, to grow and to result in recurrent disease in the months and years that follow the surgical procedure. This tumor cell entrapment hypothesis explains why there is such a high incidence of gastrointestinal cancer recurrence at the resection site and in peritoneal adhesions that surround the operative site. Unfortunately, if chemotherapy is used several weeks or months after surgery, many foci of cancer will not be treated. Neither systemic nor IP drug administration will control tumor cells in scar. Areas that were completely dissected free during the procedure will fibrose, especially those raw surfaces that make up the resection site of the primary tumor within retroperitoneal tissues. In order to prevent this recurrent cancer within the resection site and within abdominal adhesions we have employed IP chemotherapy in the early postoperative period. Figure 8 shows the pharmacokinetics of early postoperative intraperitoneal 5-FU and MMC administration. Surprisingly, the marked pharmacokinetic advantage of IP drug delivery exists in the early postoperative period as well as in the delayed post-operative abdomen.

Data gathered in our clinical trials shows that the effectiveness of intraperitoneal drug administration is greatly increased if the IP treatments begin in the early postoperative period. In our early patients in whom EPIC was not used there was a high incidence of recurrence cystadenocarcinoma in the "nooks and crannies" of the abdomen. These are sites that scar down soon after the healing process begins within the abdomen at approximately one week. Recurrent tumor in these small crevices, within abdominal adhesions, within the abdominal incision and along drain tracts has not occurred since the EPIC regimen has been employed in patients undergoing cytoreductive surgery for cystadenocarcinoma.

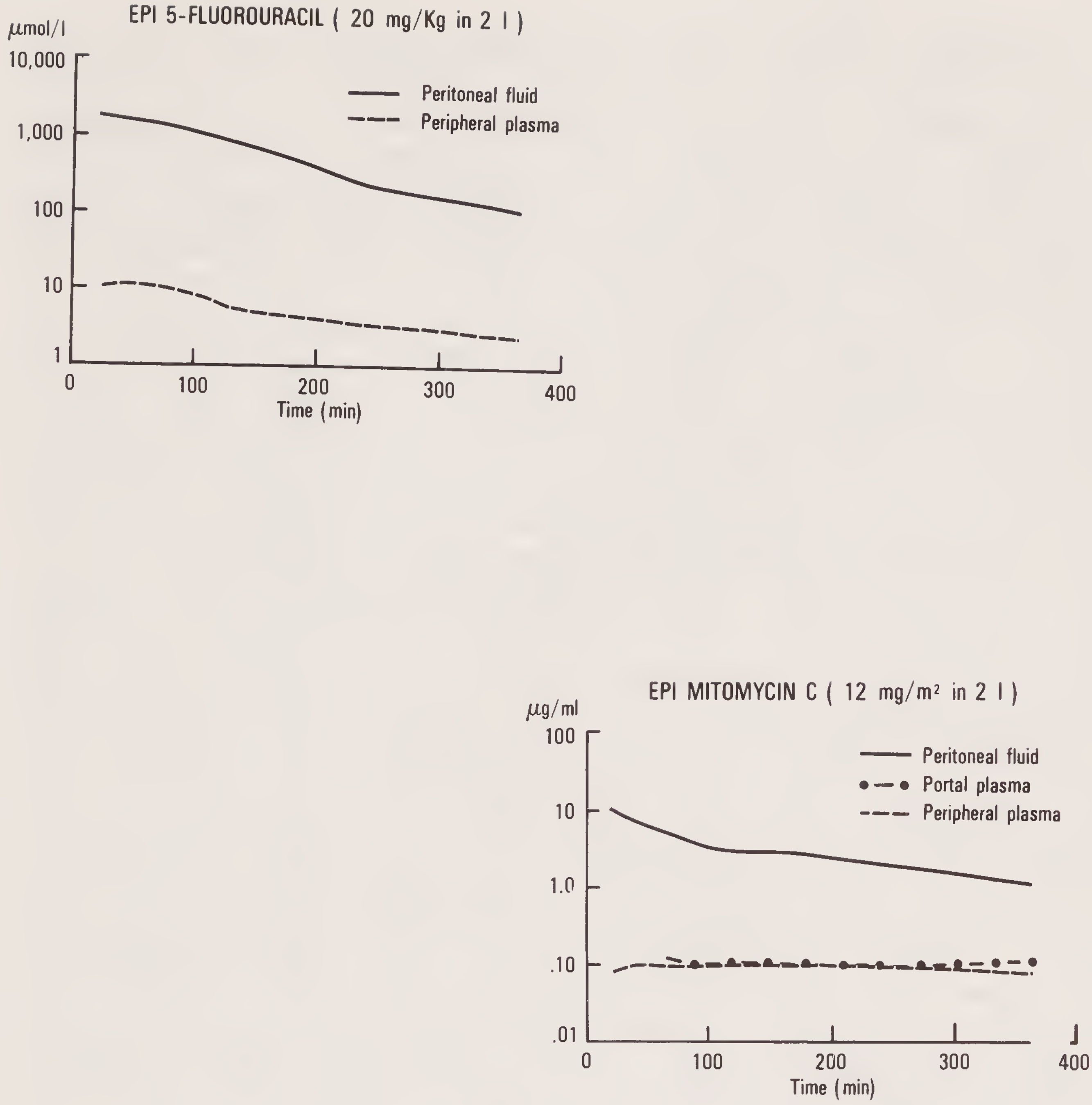


Figure 8. Pharmacokinetics of intraperitoneal 5-fluorouracil and mitomycin-C given in the early postoperative period.

CHEMOSENSITIVITY FOR COLORECTAL CANCER CELL LINES

Cancer cells can be maintained in culture short term for several weeks. A small percentage of cancers when placed in the proper *in vitro* environment will establish themselves and grow indefinitely. These phenomenon have allowed researchers to determine the exposure of a chemotherapeutic agent that is necessary to bring about the destruction of cancer cells in vitro. Figure 9 shows the chemosensitivity of human colorectal cell lines to 5-FU and MMC. These data were provided by Park and colleagues; the same levels of drug sensitivity were present for mucinous adenocarcinoma as compared to the more commonly seen colonic adenocarcinoma (8). Park found that the drug concentration with which colorectal cancer cell lines are killed with a 96 hour incubation is approximately 200 micrograms/ml for 5-FU and three micrograms/ml for MMC. These data are shown in Figure 9. When MMC is used IP the drug concentration is approximately 16 mgs/liter or 16 micrograms/ml. For MMC the drug concentration in vivo is nearly five times that which will produce cell kill in vitro. When one deals with 5-FU approximately 1500 mgs of 5-FU are placed in one liter of dialysis fluid. This is 1500 micrograms/ml. Again, approximately seven times the lethal concentration of drug is used initially for 5-FU. In our EPIC regimen the MMC exposure time is approximately 12 hours. The 5-FU is placed in the abdominal cavity for four consecutive days. This gives it an exposure time of approximately 96 hours. With this AUC one would expect a large percentage of patients to show a complete response on peritoneal surfaces. This is especially true when one considers that only minimal residual disease may be left following a cytoreductive surgical procedure (see below).

TREATMENTS

The treatment plan that we have utilized for

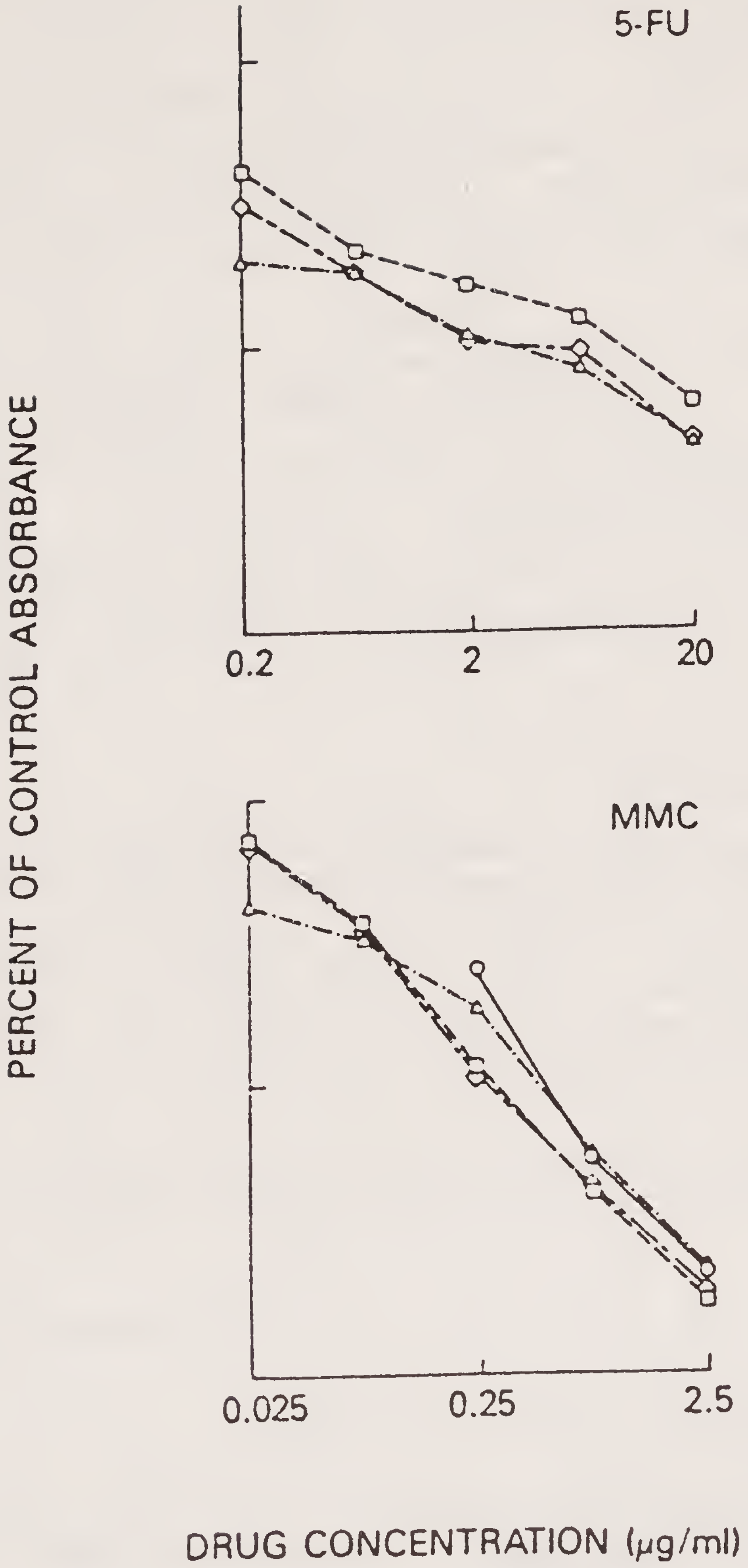


Figure 9. Chemosensitivity of human colorectal cancer cell lines to 5-fluorouracil and to mitomycin-C. A 96 hour incubation time was used.

malignant pseudomyxoma peritonei is shown in Figure 10. Because of the great bulk of the tumor seen in the majority of our patients we usually start with a cytoreductive surgical procedure (9). During this surgical procedure all gross cystadenocarcinoma should be removed. This generally involves an omentectomy-splenectomy procedure along with electrocautery resection of tumor masses from dependent portions of the abdomen. The dissection of tumor out of the pelvis can be especially difficult. Mucinous tumor lodged within the porta hepatis is safely removed using an ultrasonic dissector. If small amounts of mucoid tumor remain, especially on the small bowel surfaces, these will be adequately treated by the IP chemotherapy. However any areas of gross tumor must be resected as a part of the cytoreductive procedure if a curative approach is entertained.

On the day of surgery abdominal lavage using a standard peritoneal dialysis solution is carried out. This is to remove old blood, tissue debris and serum that would normally accumulate as a thick layer of proteinaceous material covering all the abdominal surfaces.

On the first postoperative day the initial instillation of intraperitoneal MCC at 10 mgs/m^2 is given. This is allowed to dwell of 23 1/2 hours. On the four subsequent days 5-FU is given at 15mgs/kg. A maximum dose of mitomycin-C is 20 mgs in 1 liter and the maximum dose of 5-FU is 1800 mgs in one liter.

The Tenckhoff catheter is generally removed from the abdominal cavity following the completion of EPIC.

At approximately six weeks if the patient has fully recovered from the operative event a paracentesis is performed and a soft plastic catheter is inserted into the abdominal cavity. A CT of the abdomen with IP contrast is used in order to establish the fact that there is good distribution of drug to all peritoneal surfaces. The second cycle of intraperitoneal chemotherapy is five daily instillations of 5-FU at 20mgs/kg. Usually two liters of dialysis fluid are used for the delayed postoperative intraperitoneal chemotherapy. The temporary dialysis catheter is removed after the cycle of chemotherapy is completed.

Approximately 1 month later the third 5 day cycle of IP drug instillations is given. It starts with MMC at 12 mgs/m^2 in two liters of fluid. The 5-FU is given at 20 mgs/kg for days 2-5 of the treatment. The fourth cycle of intraperitoneal chemotherapy utilizes five days of 5-FU instillations at 20 mgs/kg in two liters of dialysis fluid.

Approximately six months after the completion of four cycles of chemotherapy a staging celiotomy is performed. If any residual tumor is found at this procedure it is, of course, removed and a final cycle of EPIC with MMC and 5-FU is utilized. In follow-up patients are given CEA blood test every two months and they receive a CT scan of abdomen and pelvis at six monthly intervals. If more localized foci of tumor are discovered further cytoreductive surgery and EPIC therapy are suggested.

RESULTS OF TREATMENT

The overall results with cytoreductive surgery and intraperitoneal chemotherapy for peritoneal carcinomatosis from cystadenocarcinoma are unexpectedly good. Reference 9 summarizes our experience with this approach to peritoneal carcinomatosis. Nearly three dozen patients have been treated in protocol studies similar to that shown in Figure 10. At staging celiotomy half are found to be disease free. The other half of the patients are found to have a small volume of mucinous

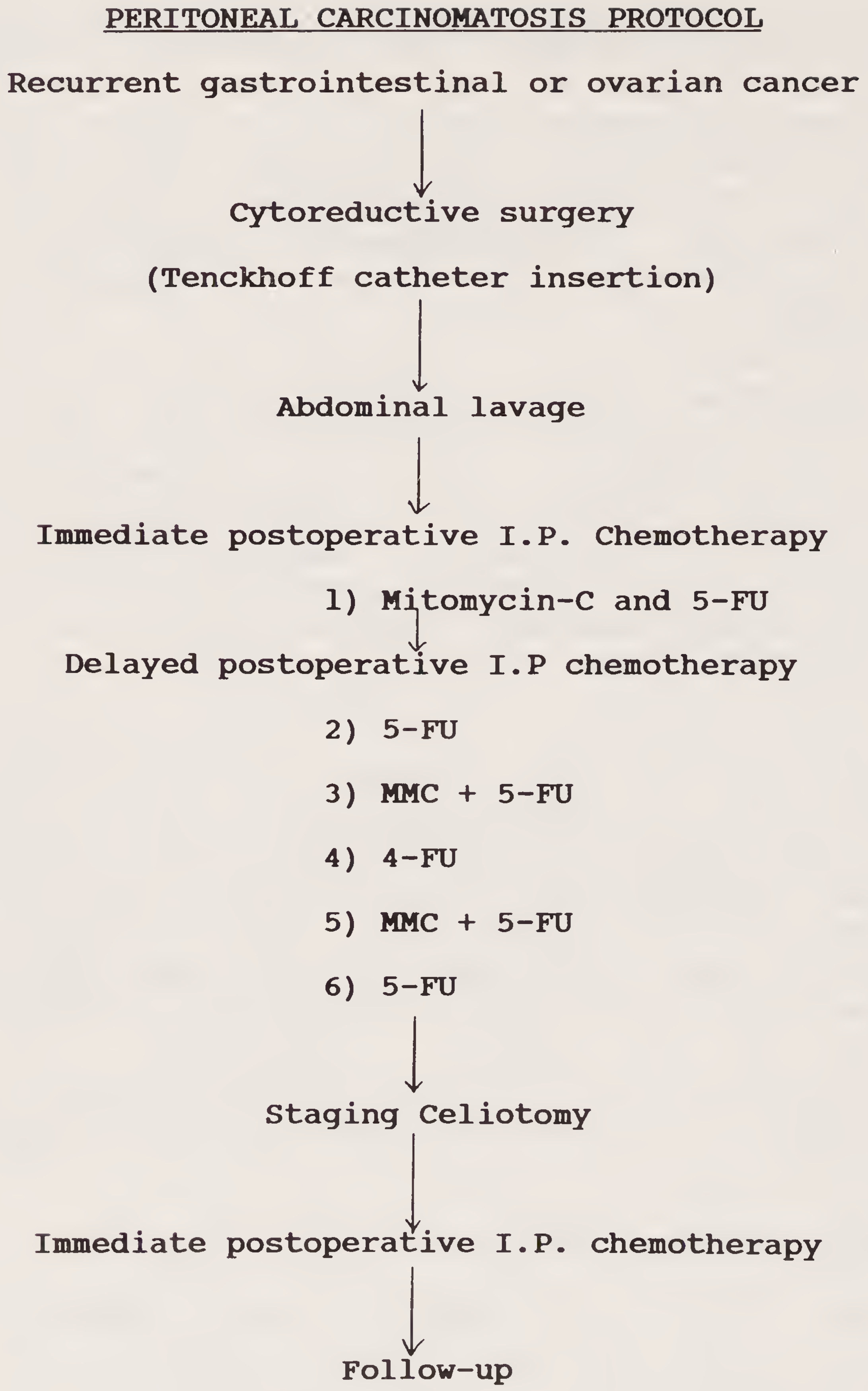

PERITONEAL CARCINOMATOSIS PROTOCOL
Recurrent gastrointestinal or ovarian cancer
Cytoreductive surgery
(Tenckhoff catheter insertion)
Abdominal lavage
Immediate postoperative I.P. Chemotherapy
1) Mitomycin-C and 5-FU
Delayed postoperative I.P chemotherapy
2) 5-FU
3) MMC + 5-FU
4) 4-FU
5) MMC + 5-FU
6) 5-FU
Staging Celiotomy
Immediate postoperative I.P. chemotherapy
Follow-up

Figure 10. Treatment plan currently utilized for malignant pseudomyxoma peritonei.

cancer. Of great interest are six patients who had cytoreductive surgery and at least three cycles of intraperitoneal chemotherapy and then came to a staging celiotomy. At surgery small quantities of residual tumor were removed and were subjected to careful light microscopic examinations. The gross and microscopic features of the tumor before treatment and after IP chemotherapy were compared. The findings are reported in Table 2. Significant histologic changes noted in treated patients included: 1) Reduction to less than 10 percent in the number of foci of atypical adenomatous epithelium. 2) Cytologic atrophy of atypical adenomatous epithelium. 3) Peritoneal sclerosis indicating chemotherapeutic effect on free abdominal surfaces. 4) Treatment effects confined to tissues in close proximity to the peritoneal cavity suggesting a direct tumoricidal effect.

The histologic changes are illustrated in Figures 11 and 12.

One may wish to reflect, at this point, as to why the systemic use of chemotherapy with colonic adenocarcinoma has tumor response rates of 20 percent or less as compared to 90 percent or greater response rates with intraperitoneal chemotherapy for cystadenocarcinoma. Most likely, this results from high sensitivity of mucinous cancer to the concentrations of intraperitoneal chemotherapy used over prolonged time periods. Also, the improved access of chemotherapy to regional sites in the early postoperative period may be of utmost importance. 5-FU and MMC intraperitoneal chemotherapy are effective in a majority of patients if adequate drug concentration over a prolonged time period are maintained.

SUMMARY

The natural history of cystadenocarcinoma of colonic or appendiceal origin was reviewed. This tumor represents a minimally invasive mucus

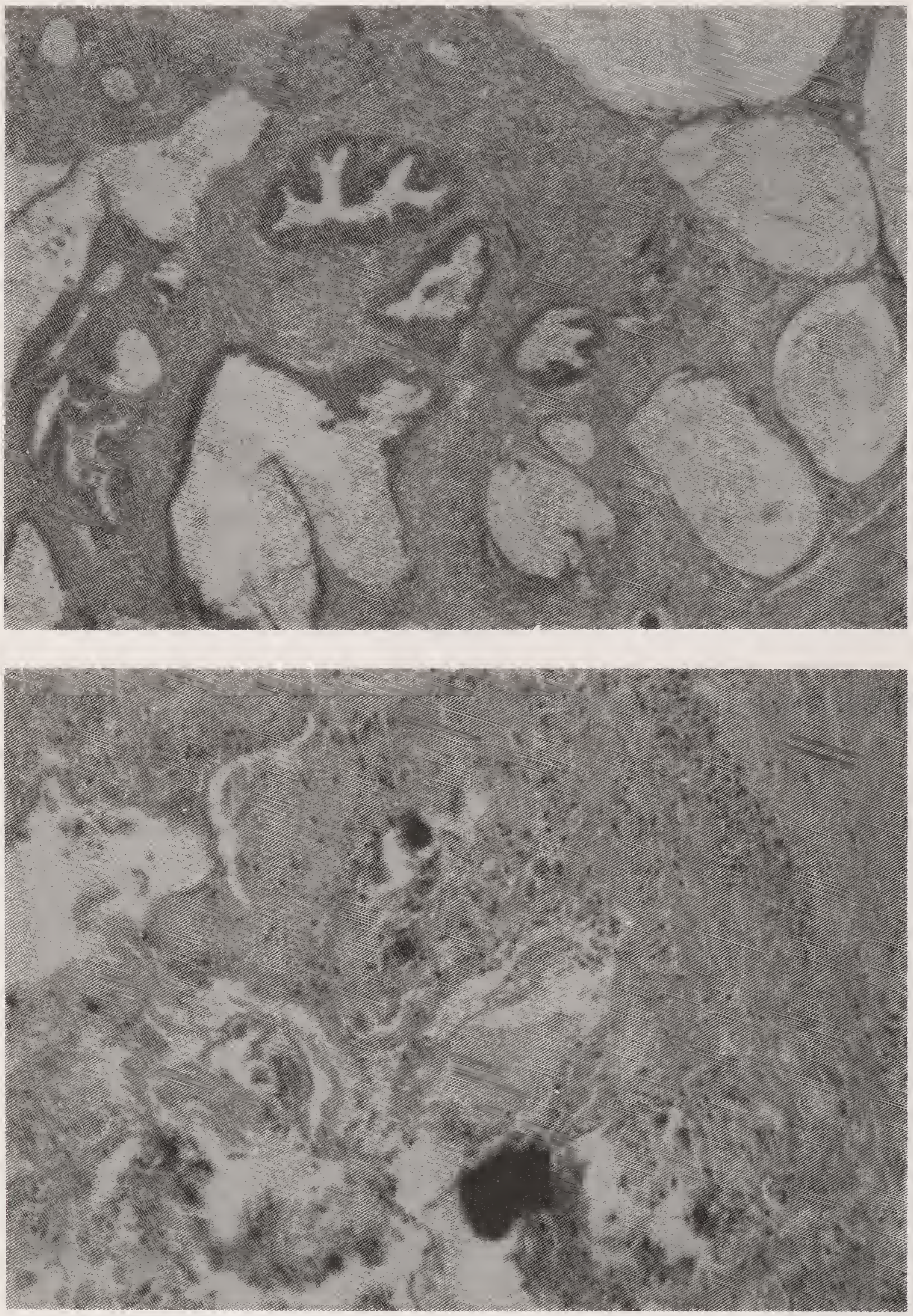


Figure 11. Top: Foci of atypical adenomatous epithelium before chemotherapy. Bottom: Tumor from the same patient recovered after intraperitoneal chemotherapy.

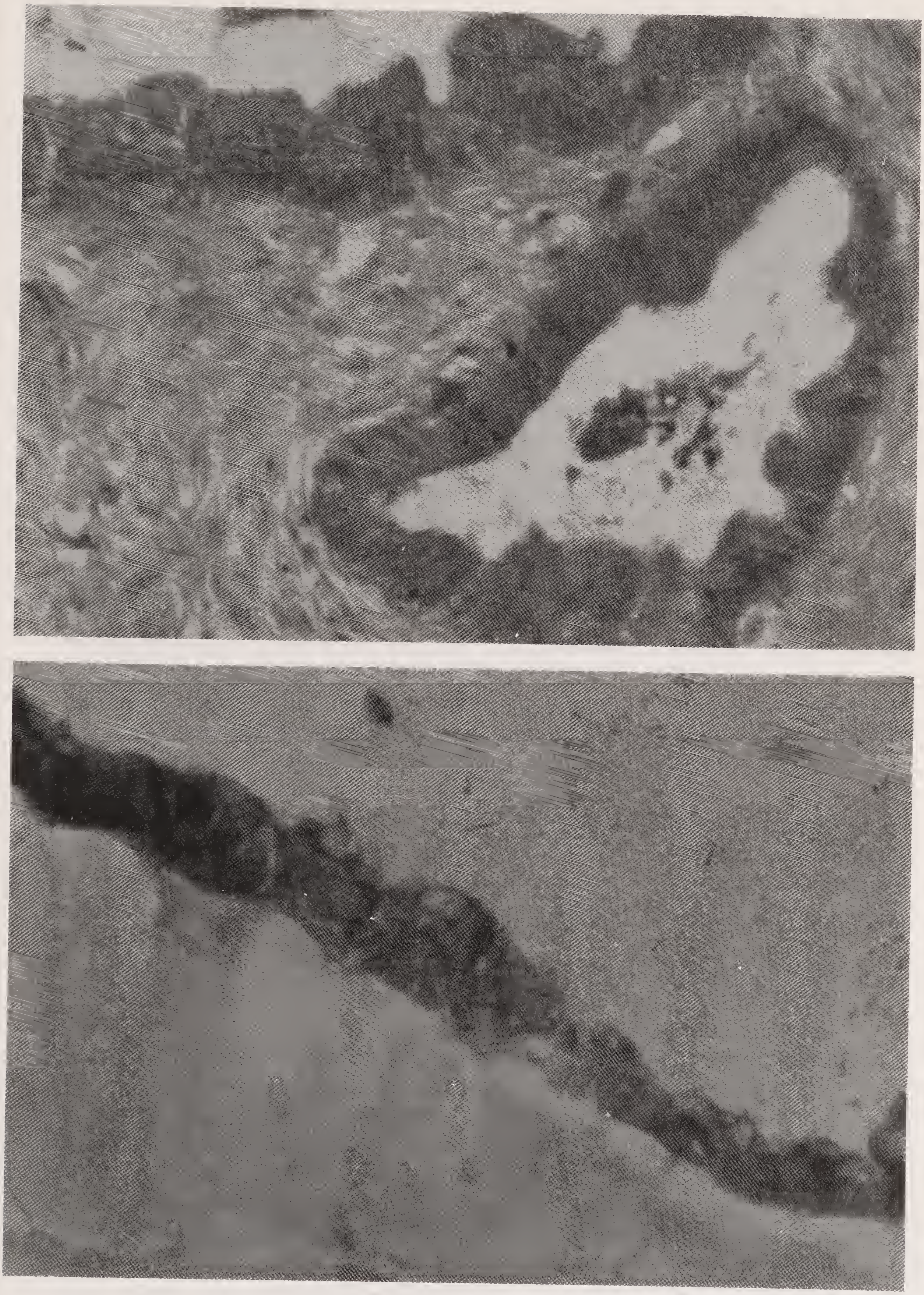


Figure 12. Top: Atypical adenomatous epithelium before intraperitoneal chemotherapy. Bottom: Atrophic area degenerating tumor cells after intraperitoneal chemotherapy.

TABLE 1
Unique clinical features of cystadenocarcinoma from colonic or appendiceal adenocarcinoma.

1. Usually presents as abdominal swelling from mucinous ascites.
2. No metastases by lymphatic channels or hematogenous routes, disseminates only by implantation.
3. Non-invasive growth of local tumor.
4. Cancer cells suspended in a water soluble matrix of mucin.
5. Tumor contained within a body cavity.
6. Relative sparing of small bowel surface so that cytoreductive surgery is possible.
7. Intestinal obstruction caused by extensive pressure on bowel lumen rather than invasion of the bowel wall.
8. Relatively slow tumor progression as compared to colonic adenocarcinoma.

TABLE 2
Histologic changes of colonic or appendiceal cystadenocarcinoma induced by intraperitoneal chemotherapy.

1. Reduction in the proportion of mucous follicles that contain atypical adenomatous epithelium.
2. Cytological degeneration and atrophy of foci of atypical adenomatous epithelium.
3. Increased calcification and inflammation.
4. Fibrose encasement of persistent tumor nodules when they are present within the treatment area.
5. Mucin lakes entirely devoid of any epithelial cells usually located within 5 mm of the peritoneal surface.
6. Peritoneal sclerosis.

producing tumor similar to what is commonly recognized within the bowel lumen as villous adenoma. This tumor is usually at an advanced stage at the time of presentation, and tends to recur at the site of tumor resection and on peritoneal surfaces. The fact that this tumor does not metastasize hematogenously or lymphatically nor does it invade locally was contrasted to its marked tendency to implant on all abdominal surfaces. The large variations in the efficiency of different types of tumor dissemination (metastases, invasion, and spread by implantation) need to be noted for this malignant process. The unique clinical features of cystadenocarcinoma were reviewed and the particular suitability of intraperitoneal chemotherapy for its treatment was discussed. Our treatment plan utilizing cytoreductive surgery and early plus delayed postoperative intraperitoneal chemotherapy was presented. The surprisingly good results of treatment was discussed. The effects of chemotherapy on tumor histology were presented in detain in six patients. Changes induced by intraperitoneal chemotherapy included a reduction in the number of foci of atypical adenomatous epithelium and marked cytologic atrophy. This plan of treatment is recommended for patients to prevent or to treat the spread of mucinous gastrointestinal cancer on peritoneal surfaces and within the resection site of the primary tumor.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR HEMATOLOGIC CANCER

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INTRODUCTION

A. Overview

The use of autologous bone marrow transplantation (BMT) to reconstitute hematopoiesis following myeloablative therapy for cancer is increasing; a recent survey indicated at least 1500 autologous BMTs would be performed during 1988 (Armitage, 1988), and this number will almost certainly increase over the next few years. Most autotransplants were performed for hematologic cancer, and this review will focus on those diseases.

B. Therapeutic Strategy

In general, autologous BMT is not performed in the untreated hematologic cancer patient, due to the beliefs that such therapy is most effective as consolidation of the "minimal residual disease" state, and that in the untreated state, a large number of tumor stem cells may be collected and subsequently reinfused. Ideally, therefore, hematologic cancer patients are treated with conventional therapy to an initial complete remission (CR). Marrow is then harvested in the usual fashion; subsequently, there are several options for its use. If the BMT is to be performed within a few days of harvest, and if the cytotoxic agents used in the conditioning regimen have been cleared, the marrow may be kept at 4°C and then infused. If more prolonged storage is required, either to allow the use of a conditioning regimen that requires more than a few days to administer, or to await a more appropriate disease state, cryopreservation at -80^{0} to $-196^{0}C$ is required. Finally, techniques to rid the marrow of occult tumor cells may be needed in some situations as discussed below.

C. Rationale

The rationale and potential advantages of autologous BMT compared to conventional therapy and allogeneic BMT may be summarized as follows:

1) A log-linear dose response curve has been observed in most drug-tumor situations (Frei and Canellos, 1980); since the use of BMT allows higher doses of cytotoxic agents to be given, it is reasonable to assume that BMT regimens kill more tumor cells than do non-BMT therapies.
2) The success of syngeneic BMT (Fefer et al., 1986) and its immunologic similarity to autologous BMT provide the basic rationale for autologous BMT. Cures of hematologic cancer patients using syngeneic BMT indicate that intensive therapy alone (presumably without an effect of graft-vs-leukemia [GVL] (Sullivan et al., 1987) that is frequently linked to graft-vs-host disease [GVHD]), can be curative for some cases in which conventional therapy is effective. Also, since GVHD is classically not present in syngeneic BMT, older patients can be treated. In sum, syngeneic BMT may serve as an idealized model of autologous BMT.
3) However, few patients have twins, and most BMTs performed to date have used marrow from normal allogeneic (usually histocompatible) donors. Moreover, while allogeneic marrow is, in theory, always preferred to autologous marrow which may have been damaged by therapy or involved with tumor, currently there are major limitations on the use of allogeneic BMT. These limitations include the fact that only 30-40% of patients have histocompatible donors, and that the immunologic problems of even histocompatible allogeneic BMT impose an age "ceiling" of 45-50 years (Klingemann et al., 1986). Furthermore, even in younger patients with histocompatible allogeneic donors, these immunologic

complications cause a mortality rate of approximately 25%; it is reasonable to assume that more complications will occur in older patients and those with less well-matched donors.

4) Finally, it is assumed that the unique potential problems of autologous BMT (i.e., damage to the stem cell compartment from previous cytotoxic therapy and re-inoculation of malignant stem cells) are soluble.

First, despite the fact that many patients who eventually receive autologous BMT have been extensively treated, engraftment can be achieved in the majority of cases, albeit with some delay (in certain situations) when compared with allogeneic BMT. It is possible that certain hematopoietic cytokines may be helpful in this regard (Peters, 1989). Of more potential importance, and despite the lack of firm evidence of its significance, the re-inoculation of malignant stem cells remains a great concern. This issue will be discussed in detail later.

CRITICAL ISSUES

A. Patient Selection

Although patient selection is critical to the success of any type of BMT, it is arguably even more important for autologous than allogeneic BMT, since the antileukemic effect of a given regimen will likely be less in the autologous situation, due both to absence of GVL and to the re-inoculation of malignant stem cells. However, while the results of autologous BMT would undoubtedly be optimized by its routine use in first CR, its use is not appropriate in first CR in situations in which conventional therapy is highly effective (e.g., childhood acute lymphoblastic leukemia, Hodgkin's disease, large-cell lymphoma, etc.). Nevertheless, it is very important to develop a schema of an optimal timing for autologous BMT for each disease. To this end, it is suggested that the latest optimal time to transplant is at the first sign of failure of potentially curative conventional therapy.

B. Conditioning Regimen

Although the importance of conditioning is universally accepted, few randomized trials of conditioning regimens have been conducted, and the resultant lack of knowledge has spawned pessimism that improved regimens can be developed. That inadequacy of conditioning is a major problem is exemplified by three considerations:

1) Rates of relapse are high with syngeneic and even allogeneic BMT, especially for most hematologic cancer patients transplanted beyond first CR (Thomas, 1983); as noted above, autologous BMT can at best produce the same relapse rates as syngeneic BMT.
2) Increasingly, more will be required of conditioning regimens, as patients transplanted after failing improved primary conventional therapy are likely to be more refractory than those who fail older regimens.
3) Most current agents permit only limited (e.g., usually 2-3 fold) dose escalation before non-hematologic toxicity becomes limiting (Herzig, 1981). This limitation is of great concern; however, autologous BMT potentially allows the use of therapies more intensive than allogeneic BMT regimens (Bearman et al., 1988), likely due primarily to the avoidance of GVHD and the effects of treatments for it.

C. Marrow Purification/Engraftment

A unique feature of autologous BMT is that relapse may potentially be due to re-inoculation of malignant stem cells. In this regard, it is likely that the type of tumor and its propensity for marrow involvement will indicate whether marrow purification is needed.

Therefore, it has been assumed that this is a vital area for some diseases, and the past decade has seen much interest and progress in developing methods to purify the marrow, removing malignant stem cells while retaining the reconstitutive potential of normal hematopoietic stem cells (Treleaven and Kemshead, 1985). Unfortunately, the utility of these techniques has been unclear, as it is currently difficult to determine whether the source of relapse is the autograft or residual tumor remaining in the patient after conditioning. This is because there is no reliable method to measure tumor stem cells in the laboratory, and because clinical trials to compare relapse rates in patients with purified vs. non-purified marrows would require large numbers of patients (Appelbaum and Buckner, 1986). In short, only where conditioning regimens are sufficiently effective to produce a very low "background" relapse rate will the effect of a given purification procedure be evaluable. As a result, it is difficult to prove that marrow purification is required for any situation. This does not, however, prove the converse--that tumor stem cells in the autograft are insignificant, as "absence of evidence is not necessarily evidence of absence". Efforts to develop laboratory assays and especially to perform these challenging clinical trials must be continued.

To date, most efforts to purify marrows have involved "negative selection"--the removal of tumor cells from the rest of the marrow; this approach (also called "purging") must in theory be 100% effective in removing malignant stem cells yet not damage normal stem cells. An alternative strategy involves removal of normal hematopoietic cells ("positive selection"). This technique has been made more attractive by the recent demonstration of engraftment by a very few normal stem cells (Turhan et al., 1989). Using this approach, it might not be necessary to collect all of the normal stem cells as long as no tumor cells were collected.

To date, however, most techniques to separate normal stem cells from malignant ones have generally used "purging" with a variety of methods, including physical (e.g., density gradients and lectins), immunologic (e.g., monoclonal antibodies plus other agents to remove cells), chemical (e.g., 4-hydroperoxycyclophosphamide [4-HC] and related compounds), and certain "biologic" (e.g., long-term marrow culture) methods. It is also recognized that combination therapy, an important clinical strategy, may be useful ex vivo as well.

CLINICAL STUDIES

A. Lymphomas and Myeloma

Hodgkin's Disease (HD). The dramatic increase in the number of autologous BMTs performed for HD (Armitage, 1988) is related to recognition that patients who fail optimal primary chemotherapy are infrequently cured, and that durable CRs have been produced even in end-stage patients by autologous BMT regimens (Phillips and Reece, 1986). As in other diseases, patient selection is critical. However, the favorable results of primary conventional chemotherapy preclude routine BMT during first CR. Conversely, some HD patients are extremely heavily pretreated and are poor candidates for such conventional therapy. Therefore, patients should be selected between these two extremes, and since patients who fail optimal primary chemotherapy are rarely cured with conventional salvage therapy, and since earlier therapy is invariably associated with better results in cancer therapy, the most appropriate time to use autologous BMT regimens in HD is at the first sign of progression after the use of optimal primary conventional chemotherapy regimens (Phillips and Connors, 1987). Certain series have shown > 50% event-free survival when such patients are so treated (Reece et al., 1988).

With regard to various conditioning regimens, many HD patients eligible for autologous BMT have previously had mediastinal radiotherapy and are thus at high risk of fatal interstitial pneumonitis (Pecego et al., 1986) with regimens that include total body irradiation (TBI). Chemotherapy regimens are therefore preferred; currently the most popular are combinations of carmustine, BCNU and VP16-213 (CBV) (Jagannath et al., 1986).

In general, purging has not been used in HD, likely due to the relative infrequency of bone marrow involvement as well as to the particular confusion about the cell of origin and therefore its removal. Moreover, we and others have noted that prior marrow involvement with HD does not exclude the possibility of durable remission using even unpurged autologous BMT, collected and frozen during a subsequent remission and reinfused at a later relapse (Phillips et al., 1989).

Non-Hodgkin's Lymphoma (NHL). NHL retains its position as the disease in which the most autologous BMTs have been performed (Armitage, 1988). Most studies have reported on intermediate- and high-grade NHL, and reveal that long-term CR can be achieved even in patients with relapsed or refractory NHL (Singer and Goldstone, 1986).

The ability of autologous BMT regimens to produce durable CR can be predicted by disease status at the time of BMT; patients in so-called "sensitive relapse" (i.e., responding to salvage chemotherapy) are clearly the most favorable subgroup. Conversely, patients with far-advanced NHL are infrequently cured even with BMT and probably should not be transplanted routinely.

If only "sensitive relapse" patients are transplanted, the question arises as to their fate with chemotherapy alone; the randomized "Parma Trial" is designed to answer that question (Philip et al., 1987). However, the main question now posed is whether there are NHL patients who should be transplanted in first CR. The answer probably is yes, but clearly only a minority of patients. For instance, the MACOP-B data from Vancouver in large-cell lymphoma patients shows a 65% event-free survival (EFS) (Klimo and Connors, 1985). Those who achieve CR (~85%) but subsequently relapse (~20%) are a very attractive group for autologous BMT during first CR, providing they can be identified reliably. Unfortunately, once these patients enter CR, relapse is difficult to predict; unless patients at very high risk to relapse are identified precisely, some patients will be transplanted when already cured (or curable) with less toxic and expensive chemotherapy. Alternatively, in high-grade NHL, certain features at diagnosis (e.g., tumor bulk) may indicate patients at high risk of relapse and for whom autologous BMT consolidation in first CR should be considered. The relatively small numbers of patients with these diagnoses preclude randomized trials.

Regarding conditioning, there is little conclusive data about even such basic matters as the requirement for TBI. Accordingly, a wide variety of such regimens are currently used (Singer and Goldstone, 1986).

The rationale for purging may be as strong for those NHLs with frequent marrow involvement (e.g., lymphoblastic and Burkitt's lymphomas) as for the leukemias. Conversely, in those diseases with only infrequent marrow contamination (e.g., large-cell lymphoma), the rationale is less clear. Data favoring purging in a heterogenous group of NHLs comes from a Dana-Farber trial (Takvorian et al., 1987), where patients received vigorous but conventional cytoreductive therapy to produce a state of "minimal residual disease". Marrow was then harvested, and purged with a monoclonal antibody (against the phenotype of the NHL) and complement preparation. After a standard chemoradiotherapy regimen, patients were then transplanted. While the results of this trial are impressive, it may be argued that they more likely indicate the benefits of using *in vivo* chemosensitivity to select a relatively favorable group of patients rather than successful *ex vivo* tumor reduction.

Myeloma. Several groups have reported autotransplant experience in myeloma; most patients were not resistant to primary chemotherapy, most received regimens that included melphalan in high dose (+/- other agents), but very few received purged marrow (Barlogie et al., 1986). Other than the observation that a few "true CRs" were produced, little can be ascertained from this experience. Nevertheless, myeloma is a disease of immense basic as well as clinical interest, and it may be anticipated that an increasing number of autologous BMTs will be performed for myeloma over the next few years.

B. Acute Leukemias

Acute Myelogenous Leukemia (AML). AML is still one of the most frequently autotransplanted diseases (Armitage, 1988), probably due to some disillusionment with post-CR chemotherapy, to the older median age of AML patients, and to the success of allogeneic BMT--especially in first CR. Since autologous BMT regimens have only rarely produced durable CR in advanced AML, the major question currently posed concerns the use of autologous BMT to consolidate first CR. This possibility is made arguable by the observation that some AML patients receiving even unpurged autologous BMT following myeloablative therapy in first CR have experienced prolonged disease-free survival (DFS) (Linch and Burnett, 1986). Also, marrow-ablative therapy and chemoseparated autologous BMT has produced durable CR in some patients with AML in second or third CR (Yeager et al., 1986), a result not usually observed with chemotherapy alone--or with autologous BMT using unpurged marrow (Yeager et al., 1986). Since randomized clinical trials will be difficult to perform in this setting, autologous BMT may at least arguably be considered as standard therapy to consolidate second CR for this group of patients. More information is required before its use during first CR can be recommended.

As with other diseases, no data clearly suggest a superior conditioning regimen. In an analysis of the European BMT Group's data (Gorin et al., 1989) indicated that TBI-containing regimens are superior to those without TBI, but the other regimen of interest, busulfan and cyclophosphamide (BUCY), was used in too few cases to evaluate.

Most purging studies for AML have used cyclophosphamide derivatives such as 4-HC. Again, studies have not been performed by methods that allow assessment of purging. However, the retrospective reviews of European BMT data (Gorin et al., 1989) indicated previously, as well the Baltimore experience (Yeager et al., 1986) seem to indicate a decreased relapse rate with chemoseparation. Given both the difficulty of proving such an effect and the acceptable level of toxicity of chemoseparation, this maneuver is likely to become standard practice.

Acute Lymphoblastic Leukemia (ALL). Many of the above considerations also apply to ALL and will not be reiterated. The main difference in the autologous

BMT experience with ALL as compared to AML is that most purging has been done with immunologic techniques in ALL and with chemical techniques in AML.

The experience of (Kersey et al., 1987) is of note, illustrating several points. These investigators compared their results using monoclonal antibody "cocktails" plus complement-purged autologous BMT with results using allogeneic BMT in a heterogenous population of ALL patients in remission. Survival rates were similar; the higher rate of toxic deaths in the allogeneic BMTs were "balanced" by the higher relapse rates in the purged autologous BMT, yielding roughly equal survivals. Interestingly, the rate of relapse in the purged autologous BMT approximated that of the allogeneic BMT patients who did not have GVHD--or presumed GVL. These results pose an interesting question about the desirability of allogeneic versus (purged?) autologous BMT.

C. Chronic Myelogenous Leukemia (CML)

Autologous BMT for CML has always been problematic (Marcus and Goldman, 1986) since complete (i.e., karyotypic) remission seldom occurs, and the stable-phase stem cells thereby employed for autologous BMT studies as treatment after transformation suggests that this approach lacks curative potential. Moreover, while the poor results noted in studies of advanced CML must be recognized as generic to advanced leukemia with BMT from any source, the rationale for using leukemic marrow for an autologous BMT in stable phase is not obvious--despite the occasional production of a Ph^1-negative state following such a transplant. However, a method to "uncover" putatively normal hematopoietic stem cells would make potentially curative autologous BMT attractive; long-term bone marrow cultures may permit such "uncovering" (Coulombel et al., 1983). This technique has recently been used in Manchester for AML and in Vancouver for CML (Barnett et al., 1989). The latter have engrafted promptly with predominantly Ph^1-negative hematopoiesis and one of three patients remains well nearly a year later with a minority of Ph^1-positive cells.

SUMMARY

In summary, autologous BMT is emerging as a way to deliver myeloablative therapy to patients with hematologic cancer ineligible for allogeneic BMT. Moreover, there are likely circumstances in which autologous is preferable to allogeneic BMT--most notably in Hodgkin's disease, but likely also in the older adult acute leukemia patient.

Work is clearly required to produce upgraded conditioning regimens, and to define the need for and utility of marrow purification procedures. However, these pressing needs should not obscure the benefits of the current use of autologous BMT techniques for selected patients--nor prevent the realization that improved patient selection is the most immediate method to improve current results.

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DOSE INTENSIFICATION USING COMBINATION ALKYLATING AGENTS AND AUTOLOGOUS BONE MARROW SUPPORT IN THE TREATMENT OF PRIMARY AND METASTATIC BREAST CANCER: A Review of The Duke Bone Marrow Transplantation Program Experience

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The treatment for breast cancer is generally considered palliative. Despite the availability of more than 15 active agents in the treatment of this disease and the ability to induce objective tumor regressions in 60-80% of treated patients, few, if any, patients are cured. Since the 1940's, the median survival for patients with metastatic breast cancer has averaged approximately 22 months, with the median time to treatment failure of most combination chemotherapy regimens which averages between 5 and 11 months. As important, complete responses in the treatment of the disease are infrequent. Most therapeutic regimens, even so-called intensive chemotherapy regimens, rarely produce complete responses in excess of 20%. Several single institution trials more recently have reported complete responses in excess of 30% and may represent important new leads in the treatment of this disease[1,2,3]. Nonetheless, the majority of patients fail to achieve complete regression of disease, and relapse and progression of their breast cancer is inevitable.

The optimal strategy for altering this situation is a subject of controversy and conceptual debate. Preclinical and laboratory experiments have suggested several therapeutic approaches that may be useful. Primary among these are dose intensification, the use of combination alkylating agents, and the treatment of early disease.

For a long time, the use of alkylating agents in combination was felt to violate certain fundamental principles of clinical pharmacology. It was generally felt that if a tumor cell was resistant to one alkylating agent, it was likely to be resistant to another; hence, the use of combination alkylating agents was not likely to afford a substantial therapeutic advantage. However, during the 1970's, experimental evidence from Frank Shabel and his colleagues at the Southern Research Institute provided convincing evidence that there was substantial non-cross-resistance among selected alkylating agents[4,5]. Surprisingly, there was as well evidence for both subadditive host toxicity and therapeutic synergy[6]. These experiments in murine models, coupled with biochemical evidence for the mechanisms underlying alkylating agent non-cross-resistance, provided the basis for subsequent extension to clinical trials.

The alkylating agents represent a very heterogeneous class of antineoplastic agents. They differ in their mode of action, methods of transport and repair. Selected agents differ substantially from one another in their primary modes of alkylation, and because of these differences, their use in combination is rational, particularly if agents are selected appropriately on the basis of their biochemical and biological properties. Griswold and his colleagues in experiments in which we collaborated have reported results of the effects of a single LD_{10} treatment with alkylating agents *in vivo* in animals bearing parental L-1210 tumor cells and cell lines which had been selected *in vivo* for resistance by repetitive treatment[7]. A single LD_{10} dose treatment will kill between 5 and 7 logs of tumor. However, a cell line resistant to cyclophosphamide (such that a single LD_{10} treatment results in tumor reduction at the end of the course of therapy) remains essentially completely sensitive to BCNU and cisplatin, and demonstrates only minor cross-resistance to melphalan and thiotepa. Similarly, a line selected for resistance to cisplatin retains essentially complete sensitivity to cyclophosphamide and carmustine. These data provide a rationale for the selection of non-cross-resistant agents in clinical trials.

A second major feature of the alkylating agents relevant to their use in high-dose therapeutic efforts is the differing non-myelosuppressive toxicity of various alkylating agents. Table 1 lists several alkylating agents and their common toxicities encountered at conventional and at escalated doses. One will note that while the alkylating agents share the common property of myelosuppression at conventional doses, at escalated doses, there is substantial variability in the toxicity encountered. This provides the scientific basis for the rational combination of alkylating agents at transplant doses: agents which differ in their non-myelosuppressive toxicity can be selected for use in combination. When these principles have been violated in clinical trials, the results have been predictable excessive organ toxicity. When tested in the clinical setting with autologous bone marrow support, we demonstrated that it was possible to combine multiple agents at full or nearly full transplant doses before dose limiting toxicity was encountered[8]. Further, the toxicity that was encountered was not predicted from the toxicities of the individual agents themselves. The combination of cyclophosphamide, cisplatin and BCNU was associated with the development of three toxicities: (1) veno-occlusive disease; (2) resistant hypertension; and (3) refractory thrombocytopenia. We have found that one could alter the organ in which toxicity is encountered by the selection of an alternate alkylating agent but that the therapeutic efficacy does not appear to be improved by such maneuvers[9]. It is possible that some modifications of the treatment regimen may lead to an improvement in the therapeutic response and a reduction of treatment-related toxicity. However, it is the belief of our group that such efforts are unlikely to make a major contribution to the understanding of the role of high-dose combination chemotherapy and autologous bone marrow support and, more appropriate at this time, is to carefully and quantitatively define the impact of such treatment programs on disease in various clinical disease settings.

Table 1

Dose Limiting Toxicity Associated with Alkylating Agent Use

Drug	Dose Limiting Toxicity Associated with Standard Dose Therapy	Dose Limiting Toxicity Associated with Therapy Utilizing Bone Marrow Support
Cyclophosphamide	Myelosuppression	Hemorraghic Myocarditis
Cisplatin	Myelosuppression	Nephrotoxicity, Neurotoxicity
Carmustine	Myelosuppression	Pulmonary Fibrosis, Toxic Hepatitis
Melphalan	Myelosuppression	Mucositis
Busulfan	Myelosuppression	Anorexia, Venoocclusive disease, Autoimmune disease
Thiotepa	Myelosuppression	Mucositis, CNS syndromes

Another basic principle underlying the development of high-dose combination alkylating agents for transplantation settings in breast cancer is the treatment of minimal and early stage disease. The recognition that tumor burdens at the start of therapy in breast cancer are in the range of 10^9 to 10^{11} cells, and the recognition that the steepness of the dose response effect for any treatment modality is shallower than with the more sensitive leukemias and lymphomas argues that treatment at smaller disease volumes may be required for successful treatment. This principle has proven valid in Hodgkins' disease, non-Hodgkins' lymphoma, and in the treatment of the acute leukemias. For a variety of biological and biochemical reasons, it is likely to be true in breast cancer. Whether the treatment of earlier disease states will provide enough of a sufficient therapeutic advantage to allow eradication of residual malignant cells is unclear and is the basis of the current investigations.

Dose Intensification

Many investigators have noted that the dose response effect for most antineoplastic agents is steep for both therapeutic and toxic effects. It may be possible, with careful selection of treatment programs, to improve the therapeutic results in breast cancer by dose intensification.

Dose intensification can be employed in two different manners: (1) "intermittent" total dose intensification can be achieved by applying a lower dose of chemotherapy continuously over an extended period of time; for example, Cooper type chemotherapy; (2) "pulse" dose intensification refers to the administration of a maximum tolerated dose over a very short period of time, allowing time for organ recovery in the interim; for example, high-dose combination chemotherapy applied in a maximum tolerated dose with autologous bone marrow support. It is possible to define most other approaches to dose intensification as variants of these two basic approaches.

The relative value of each of these methods is controversial. Most animal *in vivo* studies suggest that the optimal method of dose intensification, in terms of maximizing tumor cell kill, is to apply the dose as a single administration. Careful experimentation by Skipper, Shabel and Griswold in murine models demonstrated this effect for several tumor models and antineoplastic agents[10,11]. The effect is most prominent with the alkylating agents. The caveat is that this mode of dose intensification is also most likely to produce therapeutic toxicity in normal organs. Hence, it remains an open question which mode of dose intensification is likely to prove most successful. The potentials for combinations of various alkylating agents combined with other drugs, variations of schedule, and dose, lead to the obvious conclusion that the development of an "optimal" therapeutic strategy for treating breast cancer will be, at best, extraordinarily difficult.

Various investigators have utilized the term "dose intensification" or "high-dose therapy" in differing manners. Most investigators exploring the concept in animal or *in vitro* models use the term to refer to the total dose applied over a defined time period, particularly when utilized with repetitive treatments. In most clinical settings, the occurrence of myelosuppression or other toxic side effects of cancer chemotherapy has results in the applications of intermittent therapy, that is, chemotherapy applied on a regular basis, e.g., monthly. In order to compare different treatment schedules, Hyruniak has utilized the concept of relative dose intensity -- that is, the dose of antineoplastic chemotherapy administered over a given cycle of chemotherapy, normalized to a "standard" therapeutic regimen[12, 13]. In the case of breast cancer, this has generally been to the original "Cooper regimen". This term, however, is not generally applied to experiments involving a single dose of high-dose chemotherapy which is not to be introduced repetitively. In most studies utilizing high-dose combination chemotherapy and autologous bone marrow support, the treatment is used only once. A calculation of dose intensity in this fashion for our combination of cyclophosphamide, cisplatin and carmustine yields a relative dose intensity of 12.85 compared to the original Cooper regimen. There are other investigators who have utilized reduced doses of chemotherapy applied more than once, although the necessity for autografts in these clinical trials is questioned, and the possibility of increasing relapse through reduced dose intensity has been raised.

It is probable that the most rational definition of dose intensity will refer to pharmacologic area under the curve (AUC) or as its ultimate extension, the measurement of DNA damage induced by the particular treatment agent, and normalizing this to the treatment results obtained across various patients. It may be valuable to calculate the relative dose intensity, by using pharmacologic "area under the curve" (AUC) and maximum plasma concentration (C_{max}) as a monitor of dose intensity between therapeutic programs and among patients. The importance of this approach has been emphasized by the observation of substantial pharmacodynamic variability among patients with various drugs[14,15,16]. Unfortunately, at this time, this approach is limited to a few centers, and will likely first be used for evaluating high-dose pulse experiences or in single agent clinical trials where endpoints such as myelosuppression are easily evaluable. However, because of the relative insensitivity of most solid tumors to chemotherapy, the threshold dose for killing malignant cells, that is, the "minimum tumoricidal dose" is often close to the C_{max} achieved with standard dose therapy. For this reason, it may be that the importance of pharmacodynamic variation will be greatest in patients treated with conventional doses in which marrow supportive efforts are not utilized. Ultimately, the most precise way to define dose administration is through the use of molecular dosimetry in which direct damage occurring to normal malignant tissue nucleic acid for a given dose of alkylating agent is compared among treated patients. Although this type of approach appears to be possible with currently available techniques, it has not been extensively investigated and is technically very complex.

Our efforts over the last six years have been to systematically and quantitatively evaluate high-dose combination alkylating agents and bone marrow support to determine the therapeutic and toxic effects of this approach. We will summarize observations on over 100 patients with breast cancer treated with high-dose cyclophosphamide, cisplatin and carmustine and autologous bone marrow support. These results indicate that high-dose combination cyclophosphamide, cisplatin and carmustine with autologous bone marrow support utilized in the appropriate patient population will produce frequent and rapid complete responses which appears superior to conventional therapeutic regimens. While follow-up is yet short, there is accumulating evidence that this treatment program is effective.

Results

The therapeutic efficacy of high-dose cyclophosphamide, cisplatin and BCNU directly relates to the status of the patient at which time the treatment is initiated. Treatment of earlier stage results in higher complete response rates and improved overall therapy related results. Table 2 summarizes the objective complete responses rate for patients undergoing treatment with cyclophosphamide, cisplatin and BCNU in various disease settings. For a variety of biological and clinical reasons, we believe that the complete response rate represents a major benchmark in comparison of therapeutic regimens and that partial responses are of limited meaning. When the treatment is employed in patients who have received prior chemotherapy for metastatic disease, a high overall response rate can be obtained with an objective complete response rate of 27%. Unfortunately, these responses tend not to be durable, with a median time to treatment failure of approximately 5 months, and few, if any, patients remain in remission beyond a year.

Table 2

Responses Obtained in Patients with Breast Cancer at Differing Stage of Disease

Disease Status	Treatment Program		CR	Comments
	Induction	ABMT		
Refractory		CPA/cDDP/ BCNU	27%	*Short duration of response*
1st Relapse	-	CPA/cDDP/ BCNU	54%	*Relapse at sites of bulk disease; some durable*
1st Relapse	AFM	CPA/cDDP/ BCNU	39%	*Induction therapy only, followed immediately by high dose therapy, hence durability not studied*
1st Relapse	AFM	CPA/cDDP/ BCNU	68%	*Further follow-up required for therapeutic and toxic effects*
1st Remission; Stage II, 10+ LN high risk adjuvant setting	CAF	CPA/cDDP/ BCNU	N/A	*Pilot study now with 29 patients enrolled.*

Abbreviations: CPA - cyclophosphamide; cDDP - cis-diaminodichloroplatinum; BCNU - carmustine; AFM - adriamycin, 5-fluorouracil, methotrexate; CAF - cyclphosphamide, adriamycin, 5-fluorouracil; 1st relapse - patients with recurrent metastatic breast cancer who have not received prior chemotherapy for metastatic disease.

An improvement in the complete response rate can be obtained by treating patients who have received no prior chemotherapy for metastatic disease. Fifty-four percent of patients treated with cyclophosphamide, platinum and BCNU and autologous marrow as the initial chemotherapy for metastatic disease obtained objective complete response[17,18]. Again, the majority of these patients relapsed rapidly, but approximately 15% of the patients have remained in continuous, unmaintained remission beyond two years. Relapses occurred predominantly at pretreatment sites of bulk disease or in areas of prior radiation therapy. These observations suggest that there is a limit to the therapeutic efficacy of this treatment program, and that large tumor burdens will need to be reduced prior to application of high-dose therapy as consolidation. A third unique feature of the treatment of patients with high-dose combination alkylating agents as initial treatment for metastatic disease is the observation that the achievement of a complete remission was rapid, with the median time to CR in those patients attaining a CR of 18 days. Finally, and important for the development of this overall approach, is the toxicity of this treatment program is significant -- therapy related death occurred in 22% of treated patients. Patient fatalities tended to occur when there were large tumor burdens, and particularly in patients where there was extensive replacement of a normal visceral organ by malignancy. Careful patient selection and efforts to reduce treatment related toxicities through the use of recombinant growth factors and other strategies may reduce the treatment related mortality in this patient population.

The recognition of relapse at pretreatment sites of bulk disease and the availability of active agents in breast cancer that were not included in the transplant regimen suggested that an induction course of chemotherapy might reasonably be combined with the high-dose cyclophosphamide, cisplatin and BCNU in extending the therapeutic utility of this program. Consequently, an induction treatment program entitled "the Duke AFM Program" was developed[19]. Adriamycin, fluorouracil and Methotrexate are used intensively two to four cycles until the best response or complete response is obtained. At this point, consolidation using high-dose cyclophosphamide, cisplatin and BCNU and autologous bone marrow support is used to consolidate the regression achieved with AFM. Surgery to pretreatment sites of bulk disease or the addition of radiation therapy to consolidate areas for pretreatment bulk disease is then employed. This treatment program has yielded an overall complete response rate of 68%. Patients achieving a partial response will generally not remain in remission for an extended period of time. However, of the patients who have achieved a complete response, only 15% of patients have relapsed. Further follow-up is clearly required since the median follow-up at this point is only 11 months. However, the high frequency of complete response and the durability thus far of unmaintained remissions is encouraging.

The finding of a high complete response rate, both to the induction regimen of Adriamycin, 5-fluorouracil and methotrexate, and the apparent ability of high-dose therapy to achieve effective consolidation has led us to undertake randomized comparative trials of high-dose consolidation with cyclophosphamide, cisplatin and BCNU and autologous bone marrow support versus observation until relapse followed by treatment with the same high-dose regimen and autograft in patients who achieve a complete response to Adriamycin, fluorouracil and methotrexate. This trial is in progress and careful follow-up of this patient group will be important for evaluating the final utility of high-dose therapy and autografting in the treatment of breast cancer.

The prognosis for patients with Stage II breast cancer which has involved ten or more axillary lymph nodes is extremely poor. The median time to relapse, despite adjuvant chemotherapy, is one year, with a median survival of 3.4 years. Clinical trials with adequate follow-up suggest that disease-free survival for these patients is less than 20%, and in most centers, less than 10%. Because of this high risk of relapse, we have undertaken a prospective pilot trial of 4 cycles of cyclophosphamide, adriamycin and 5-fluorouracil followed by consolidation with high-dose cyclophsophamide, cisplatin and BCNU with autologous bone marrow support. To date, no systemic relapse has occurred. Local relapses have occurred in two patients in the surgical scar, and radiation therapy has now been added as adjunct in this area. Full patient accrual and further follow-up will be required.

Discussion

These sequential quantitative studies utilizing a single regimen in the treatment of breast cancer suggest certain conclusions. The use of high-dose therapy in advanced disease in previously treated patients will produce frequent responses, but such responses are not durable. This is probably due to large tumor burdens and the selection of resistant cell populations by chemotherapy. Further, treatment with high-dose chemotherapy programs is likely to be less well tolerated in patients who have received prior chemotherapy.

The use of high-dose chemotherapy with autologous bone marrow support appears to be a valuable tool in the treatment of early metastatic disease, particularly combined with an intensive induction regimen. The ability to produce substantial complete regressions may translate into improved overall outcomes. Further follow-up of this patient population will be required.

Finally, the logical extension of intensive treatment approaches is the use in high-risk adjuvant settings. The ability of cyclophosphamide, cisplatin and BCNU, particularly when coupled with an effective induction regimen that produces substantial responses in advanced disease, provides hope that this therapeutic approach may provide adequate cytoreduction in this selected patient population. Again, further follow-up will be required.

A major issue confronting the application of this therapeutic approach is the toxicity associated with high-dose therapy. The use of recombinant myeloid growth factors to accelerate hematopoietic recovery has, in our hands, been associated with a reduction in treatment-related morbidity and mortality. GM-CSF[20] and G-CSF[21] have been demonstrated to enhance myeloid recovery, leading to a shortening of the myelosuppression and an associated reduction in bacteremias and treatment-related toxicity. Hepatic dysfunction and renal dysfunction in patients that have received recombinant growth factors have been substantially reduced[22]. Further, compared to historical controls not receiving recombinant growth factors, treatment-related mortality has also been reduced. Randomized comparative trials to evaluate the importance of these effects will need to be undertaken.

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DOSE INTENSITY STUDIES IN BREAST CANCER - AUTOLOGOUS BONE MARROW TRANSPLANTATION

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It has been stated by a number of authors that the chemotherapy of breast cancer has reached a plateau and that no further improvement in results was possible with currently available treatments (Lippman, 1987; Tannock, 1988). However, even a cursory review of the literature shows broad differences in response rates and survival durations for patients with metastatic breast cancer (Hryniuk, 1984). Although many of these differences might be attributed to patient selection and differences in the distribution of known prognostic factors, and in the case of response rate to differing criteria for response evaluation, we will concentrate on the dose intensity of chemotherapy as a variable, that, as opposed to other prognostic factors, can be controlled and modified by the treating physician.

The evidence from experimental systems conclusively shows that cytotoxicity of antitumor agents over tumor and host cells is clearly dose related (Bruce, 1966; VonHoff, 1986; Schabel, 1984). Although the slope of the dose/response curve varies with the different cytotoxic agents, in general, the higher the dose, the higher the antitumor effect measured by cytotoxicity. However, since a single dose of chemotherapy is seldom curative in human malignancies, the intensity of one dose is just one component of a successful antitumor therapy. The other important treatment-related component is the frequency of administration. The two combined define the dose-rate. There is convincing evidence from radiobiology that the dose rate is an important variable in the curative effect of ionizing radiation (Withers, 1988). In this report we will review our clinical studies with chemotherapy for metastatic breast cancer at our institution designed to assess the importance of dose rate, or dose intensity, and its correlation with the outcome of therapy.

Dose Rate in Standard Combination Chemotherapy

Between July 1973 and June 1976, 694 women with metastatic breast cancer were treated with various chemotherapy protocols of the Medical Breast Service of this institution. All chemotherapy protocols utilized during that time included doxorubicin and cyclophosphamide, and either fluorouracil, ftorafur or vincristine. A retrospective analysis, including extensive evaluation of prognostic factors, showed these various regimens to be therapeutically equivalent (Swenerton, 1979). Over 50 pretreatment host, tumor or treatment related characteristics were reviewed, and correlated with response to therapy and survival. Extent of prior treatment, performance status, extent of metastatic disease, and integrity of organ function, as defined by various biochemical and hematologic studies, were closely correlated with response rate and survival. Other characteristics, such as age, menstrual status and the location of metastases, did not correlate with either end point. The dose of chemotherapy was evaluated utilizing as standard the intended protocol dose. As shown on Table 1, those patients who received the intended dose of chemotherapy, or a higher dose (since dose escalation clauses were included in all protocols) had a higher

Table 1. Influence of Dose and Frequency of Chemotherapy Administration on Response Rate and Survival

	No. of Patients	No. with Response (%)	P	Median Survival (wk)	P
Given dose[1]					
≥100%	182	125 (69)		112	
80-99%	309	204 (66)	0.01	89	0.19
<80%	128	68 (53)		84	
Interval Between Cycles					
≤21 days	367	265 (72)		103	
22-28 days	185	112 (61)	0.01	91	0.33
>28 days	41	19 (46)		76	

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[1] Expressed as a percentage of intended dose adjusted to body surface area.

response rate, and longer survival, than patients treated with lower doses. There seemed to be a linear correlation of dose with response, although at the range of doses used, the slope of the curve was not very steep. The correlation of dose with response rate was highly statistically significant, while the correlation with survival showed a trend which did not reach statistical significance.

We also evaluated by univariate analysis the effect of frequency of administration of chemotherapy on response rate and survival. Those patients who received chemotherapy more frequently (every three weeks) had a higher response rate, and longer duration of survival, than patients who received chemotherapy less frequently. In this correlation, too, the correlation with response rate was highly statistically significant while that relating to survival showed only a nonsignificant trend. Although multivariate analysis of this prognostic factor study was performed later, neither dose, nor frequency of administration were included, since our goal at the time was to evaluate pretreatment characteristics. A similar dose-response correlation was observed in a retrospective analysis of our adjuvant chemotherapy trials with the same type of chemotherapy (Buzdar, 1984).

The overall response rates achieved with standard chemotherapy in these studies ranged from 60-70% with median durations of survival ranging from 76-99 weeks. The dose limiting toxicity of the chemotherapy regimens in these trials was myelosuppression. While these chemotherapy studies were ongoing, protected environments, and prophylactic antibiotic regimens became available. Since we could deal more effectively with the complications of severe myelosuppression, we decided to evaluate prospectively the effect of dose intensity on response rate and survival in patients with metastatic breast cancer.

Randomized Trial of Standard Versus High-Dose Combination Chemotherapy

At the time this study was designed we considered the FAC combination the best regimen available. Today, ten years later it remains the best standard chemotherapy program. In a prospective, randomized trial we wanted to compare the efficacy of standard FAC with the highest tolerable dose of the same combination, used as a short induction regimen (3 cycles), followed by a maintenance regimen consisting of FAC at standard doses, and subsequently, CMF. The dose of doxorubicin in the high dose combination was 40-100% higher than the standard dose whereas the doses of cyclophosphamide were 140-260% higher than standard doses (Table 2). The dose of 5-fluorouracil was 150% higher than that used for standard FAC. However, since 5-fluorouracil was administered by a five-day continuous infusion, a pharmacokinetic analysis suggested that the dose-intensity of this "high dose" regimen of 5-fluorouracil was, in fact, lower than that of bolus 5-fluorouracil administered as part of standard FAC. The results of this trial have been described elsewhere (Hortobagyi, 1987). The pretreatment characteristics of the control and high dose chemotherapy groups were evenly distributed. The overall remission rates were identical (78% in both groups) and so were the complete remission rates. In addition, the durations of response (11 and 10 months) and survival (20 months for both groups) were also similar, suggesting that neither regimen produced a better antitumor effect than the other. However, the analysis also showed that complete responses were obtained faster in the high dose chemotherapy group (median time to complete remission 8 versus 22 weeks). The toxicity of the high-dose regimen was considerably higher, making standard FAC a better choice. Subsequent to the completion of this trial Dr. Hryniuk and his collaborators developed a better method to evaluate dose intensity

Table 2. Chemotherapy Doses for Patients Treated In and Out of a Protected Environment

Drug	Dose (mg/m^2)/Route/Day of Cycle		
	Cycle 1	Cycle 2	Cycle 3
Protected Environment			
5-Fluorouracil	500 IV (continuous infusion, daily for 5 days (d1-5)		
Doxorubicin	70 IV d 1	85 IV d 1	100 IV d 1
Cyclophosphamide	1,200 IV d 1	1,500 IV d 1	1,800 IV d 1
Control			
5-Fluorouracil	500 IV d 1 & 8	500 IV d 1 & 8	500 IV d 1 & 8
Doxorubicin	50 IV d 1	50 IV d 1	50 IV d 1
Cyclophosphamide	500 IV d 1	500 IV d 1	500 IV d 1
CMF Maintenance			
Cyclophosphamide	600 IV d 1		
Methotrexate	50 IV d 1 & 8		
5-Fluorouracil	600 IV d 1 & 8		

Abbreviation: IV, intravenous.

(Hryniuk, 1984). We decided to apply this method retrospectively to the randomized trial of standard versus high dose FAC to assess why, despite the seemingly much higher doses of chemotherapy during the induction phase no therapeutic benefit was observed over standard FAC. Our analysis showed that standard FAC had a 0.97 dose intensity compared to the CAF regimen described by Bull et al, and used by Hryniuk as standard in his previous publications. The high dose FAC had a planned dose intensity of 1.33 during the initial three cycles, and 0.97 thereafter. The administered dose intensity of the high dose and standard regimens was 1.32 and 0.90 during the first nine weeks of therapy, but by the end of 24 weeks the administered dose intensities had decreased to 0.81 and 0.75, respectively. The advantage in dose intensity gained during the initial nine cycles was lost later because standard chemotherapy could not be administered at the planned dose or frequency of administration after high-dose induction. Presumably this occurred because the initial high doses of chemotherapy produced bone marrow damage that resulted in poor tolerance to standard doses of chemotherapy. Our conclusions from that study were that extramedullary toxicity of high dose FAC chemotherapy was too high, and that the differences in dose intensity, too modest, for any real differences in therapeutic benefit. While this study was going on autologous bone marrow harvesting and transplantation were being developed at our institution. By 1983 it was clear that this technique allowed the relatively safe administration of very high doses of cytotoxic agents, by shortening considerably the duration of severe myelosuppression (Spitzer, 1984). Utilizing autologous bone marrow, the dose intensity of several drugs could be increased by 10-20 fold depending on the cytotoxic agent utilized.

High Dose Chemotherapy with Autologous Bone Marrow Rescue

By 1983 several studies had shown the efficacy of high-dose chemotherapy in combination with autologous bone marrow support in inducing high overall remission, and complete remission rate in patients with Hodgkin's disease, non-Hodgkin's lymphoma, neuroblastoma, and other solid tumors. Some patients treated with only one course of high dose combination chemotherapy were in a long-term unmaintained complete remission. Information from several centers showed that high-dose single agent, or combination chemotherapy produced high response rates in metastatic breast cancer refractory to standard therapy (Antman, 1988). However, the duration of such responses was short. We elected to explore the use of high dose chemotherapy with autologous bone marrow rescue not as induction therapy but as intensification therapy following standard induction chemotherapy (FAC) regimens. We selected the group of patients with estrogen receptor-negative, or hormone refractory, metastatic breast cancer since these patients, although they respond to chemotherapy with equal frequency to their estrogen receptor-positive or hormone dependent counterparts, they remain in response for shorter periods of time and their survival is less than half as long as that of patients with hormone dependent tumors (Osborne, 1987). Therefore the trial was designed for patients with measurable metastatic breast cancer, <65 years of age, good performance status and normal organ function, and without prior chemotherapy. These patients were treated with 3-6 cycles of combination chemotherapy that included doxorubicin, and cyclophosphamide, and autologous marrow was harvested during the administration of induction chemotherapy. The techniques of marrow collection, storage and thawing have been described elsewhere (Spitzer, 1984). We elected a three-drug combination for consolidation consisting of cyclophosphamide, etoposide and cisplatinum. Both cyclophosphamide and cisplatinum were known to be effective against breast cancer, and there was preclinical information suggesting that etoposide enhanced the cytotoxic activity of the alkylating agents. In addition, all three drugs could be escalated substantially and, with the exception of cisplatinum, there was little extramedullary toxicity. Also, our Bone Marrow Transplantation Team had extensive experience with the CEP combination, in use for similar strategies in different tumor types. The plan was to administer two cycles of high-dose chemotherapy back to back, the second given upon recovery from toxicity caused by the first one.

Cyclophosphamide was administered at doses of 4.5-5.25 gm/^{2} in three divided doses, etoposide at 750-1200 mg/m^2 in three divided doses and cisplatinum at doses of 120-180 mg/m^2 per cycle.

Patients were hydrated continuously with 5 liters of intravenous fluids per day during the administration, and until one day after the completion of high dose chemotherapy to diminish both the risk of cyclophosphamide bladder toxicity, and cisplatinum nephrotoxicity. Those patients scheduled for autologous bone marrow transplant received their marrow 2-3 days after the third, and last day of high dose chemotherapy. Patients were treated in private hospital rooms, or in a germ-free isolation unit. Oral prophylactic antibiotics were administered, and appropriate intravenous antibacterial therapy was given when patients became febrile. If needed, irradiated blood products were administered.

Fifty-four patients with metastatic breast cancer (47 of them evaluable) have been treated with this strategy (Spitzer, 1988). All had estrogen receptor-negative, or hormone refractory metastatic breast cancer. The median age was 40 years (range 29-62). Seventy-two percent of the patients were pre- or perimenopausal, as would be expected by the limitations in age, and hormone responsiveness.

Among the evaluable patients 16 had one site of metastases before starting induction chemotherapy while the other 31 had two or more sites of metastases. Since patients with bone marrow invasion or extensive bone involvement were excluded the sites of metastases were distributed as follows: soft tissue in 36%, lymph nodes in 53%, lung or mediastinal metastases in 53%, bone metastases in 36%, and liver metastases in 21%. The median disease-free interval from diagnosis was 5 months (range: 0-76 months). The response to induction FAC therapy was as follows: complete remissions were obtained by 30% of patients, and partial remissions by 50%. After completing induction FAC and high dose chemotherapy intensification 58% of those patients were in a complete remission. The median progression free interval from induction therapy was projected to be 55 weeks, with a projected two-year progression-free survival of 35%. Four patients have been followed in a complete remission beyond two years. The median survival from induction is estimated at 111+ weeks while the median survival from the first cycle of intensification is estimated at 98+ weeks.

Toxicity of this high-dose chemotherapy regimen included moderate nausea and vomiting in 60% of patients, and febrile episodes, including documented infections, in 76% of patients. Stomatitis, diarrhea, and cisplatin-induced neurotoxicity have occurred in a smaller percentage. There have been four deaths, one of an acute myocardial infarction, two due to sepsis and one to ARDS.

Approximately half the patients treated on this trial received high dose chemotherapy without autologous bone marrow transplantation. This was done as part of a prospective randomized trial to assess (a) the need of autologous bone marrow and (b), whether the administration of bone marrow potentially contaminated by autologous tumor cells might alter the effect of high dose chemotherapy. Most patients recovered their peripheral blood counts promptly, with or without bone marrow infusion, although after the second course the duration of myelosuppression was somewhat prolonged for patients who did not receive their harvested bone marrow (Dunphy, 1988). There are no differences, thus far, in the duration of response of the groups treated with and without autologous bone marrow support.

In an attempt to develop a second, and noncross-resistant high dose chemotherapy combination we initiated a couple of years ago a phase I-II study of a combination of mitoxantrone, etoposide and thiotepa. The trial was open to patients with metastatic breast cancer with progressive disease after frontline chemotherapy. Good performance status, normal organ function, and age under 65 were eligibility requirements. Mitoxantrone was administered at 30 mg/m^2, etoposide at 1200 mg/m^2 in three divided doses and thiotepa at 750 mg/m^2 also in three divided doses (MET). Autologous bone marrow was reinfused 7-9 days after the initiation of therapy.

Most patients were heavily pretreated; the median number of prior chemotherapy combinations was three (range = 2-8), and 18 of them (67%) had progressive disease at the time of high dose chemotherapy with MET. The median age of the entire group was 42 years (range = 16-57 yrs). The disease-free interval from diagnosis was 58 weeks (range = 0-217), with 69% of patients presenting with estrogen receptor negative metastatic breast cancer. Fifty-three percent of patients were premenopausal.

Thirty-two patients have been treated with at least one cycle of this combination, and 13 of them received a second cycle of high dose chemotherapy. The median follow-up is 16 weeks. Of the 28 patients evaluable for response, there have been seven complete remissions and an overall remission rate of 79%.

Of the seven patients who achieved complete remission five had progressive disease at the time of high dose chemotherapy. Five of the complete remission patients remained disease-free 10-43 weeks after high dose therapy. The median progression-free survival from the initiation of MET for the entire group of patients was 15 weeks, but it has not been reached for the patients who achieved a complete remission.

Toxicity

Grade 3 or 4 mucositis was more frequent with this combination (69%), and infections were also common. Documented sepsis was observed in up to 30% of patients. There were three early deaths related to infection during neutropenia, ARDS and congestive heart failure, respectively: all three patients had been heavily pretreated with anthracyclines administered by bolus.

Comment

The studies reported in this paper suggest that dose intensity is a complex concept. In addition, the correlation of dose-intensity with outcome is difficult to demonstrate in the human breast cancer model. Although retrospective review of planned or administered dose-intensity in patients receiving "standard" combination chemotherapy suggests the presence of a dose response correlation, there are many confounding variables to be taken into consideration including performance status, extent of metastatic disease and treatment-related toxicity. The concept of dose-intensity will need continued evaluation prospectively, and with careful consideration of other known prognostic factors, and their interactions.

Our studies with intensified FAC regimens were disappointing because they failed to produce an improved therapeutic index. However, they taught us that not all drugs may be appropriate for intensification in the traditional sense, unless and until we develop means to control, and reverse the toxicity related to increased doses. These trials also demonstrated that a modest increase in dose-intensity for a short period of time was insufficient to alter the natural history of advanced breast cancer. Modest dose intensification for a more prolonged period of time could now be studied with hematopoietic colony stimulating factor support, a strategy currently under study in clinical trials.

The current generation of high dose chemotherapy regimens with autologous bone marrow support has produced encouraging results in our hands and elsewhere (Antman, 1988; Spitzer, 1988; Peters, 1988). Clearly, despite the opinion of some skeptics (Henderson, 1988), the response rates and, especially the complete response rates obtained with this strategy have been consistently better than those obtained with standard chemotherapy for metastatic breast cancer. It is unclear at this point whether high-dose chemotherapy used as induction therapy, or high-dose chemotherapy used as consolidation is the best strategy to follow, and both strategies need to be evaluated further. The initial high mortality rate with these regimens has been decreasing with additional experience thus further improving the risk: benefit ratios. From the available information it is unlikely that a single cycle of currently available high-dose combinations will result in a major change in cure rate of metastatic breast cancer. Multiple doses of high dose chemotherapy, or regimens incorporating multiple active drugs in metastatic breast cancer will need to be studied, preferably in controlled trials comparing them to the administration of standard chemotherapy. This would definitely assess their relative efficacy in advanced disease. Our analysis of prognostic factors in the high dose chemotherapy program suggested that patients with minimal or small amount of metastatic disease, good performance status and long disease-free survival were more likely to obtain a durable progression-free survival after high-dose chemotherapy than patients with more extensive disease, or poorer performance status. In addition, the latter were more likely to experience severe, and life threatening toxicity.

As the studies in metastatic disease mature, we will need to evaluate these strategies in high-risk patients with primary breast cancer (stage III, inflammatory breast cancer, etc) to assess the curative potential of these treatments.

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USE OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) TO SUPPORT INTENSIVE CHEMOTHERAPY.

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GM-CSF is one of the four hematopoietic growth factors (Table 1) which is available in recombinant form and several phase I and II clinical studies have shown that it can increase the leukocyte count in the blood in a dose dependent fashion under various clinical conditions (Klingemann,1988). Three different systems are currently being used for the biosynthetic production of GM-CSF: 1) mammalian cells, 2) yeast and 3) E. coli. Since the E. coli derived material is less glycosolated than the yeast product, a lower dose is usually required for E. coli derived GM-CSF. It is also believed that because of the differences in molecule size, the tissue penetration and distribution pattern for E. coli GM-CSF is different.

GM-CSF has been given for various clinical conditions (Table 2), and the observations from these phase I/II studies allow for the following conclusions:

* In bone marrow failure (e.g. aplastic anemia), GM-CSF is effective as long as a certain hematological reserve is available (Champlin et al., 1989; Nissen et al., 1988).

* GM-CSF by itself has a limited ability to stimulate other hematopoietic cell lineages, such as megakaryocytopoiesis and erythropoiesis.

* GM-CSF increases the neutrophil count in a predictable and dose dependent manner in patients with myelodysplastic syndrome (Ganser et al., 1989; Vadhan-Raj et al., 1989) and AIDS related neutropenia (Groopman et al., 1987).

* GM-CSF shortens the period of neutropenia after

chemotherapy and treated patients have a higher nadir.

* GM-CSF is suitable to accelerate engraftment in patients after bone marrow transplantation (Brandt et al., 1988; Nemunaitis et al., 1988).

Table 1 Human Colony-Stimulating Factors

Colony-Stimulating Factor	Source	Cell Maturation
GM-CSF	T-lymphocytes Endothelial cells Fibroblasts Macrophages	Neutrophils, Monocytes, Eosinophils
G-CSF	Mononuclear Phagocytes Fibroblasts	Neutrophils
IL-3	T-lymphocytes	Early progenitor cells in bone marrow
M-CSF	Endothelial cells Fibroblasts Placenta	Monocytes

Table 2 Diseases In Which GM-CSF Has Been Given

* Aplastic anemia
* Radiation Accidents
* Myelodysplastic syndrome
* After chemotherapy
* After bone marrow transplantation
* AIDS induced neutropenia

One of the critical issues related to the administration of GM-CSF is concerned with the question of whether or not GM-CSF can stimulate growth of leukemic and other malignant cells. For myeloid cell lines, Delwel et al. (1988) have shown by blast colony formation and DNA synthesis studies that about 60% of all tested cell lines proliferate if exposed to GM-CSF. For lymphoblastic cell lines, no consistent reports exist. Occasional observations, however, have indicated that this possibility should at least be taken into consideration (Santolini et al., 1987; Uckun et al., 1988). Based on these results, treatment of myeloid leukemias with GM-CSF should be limited to compassionate indications such as graft failure or delayed engraftment after bone marrow transplantation.

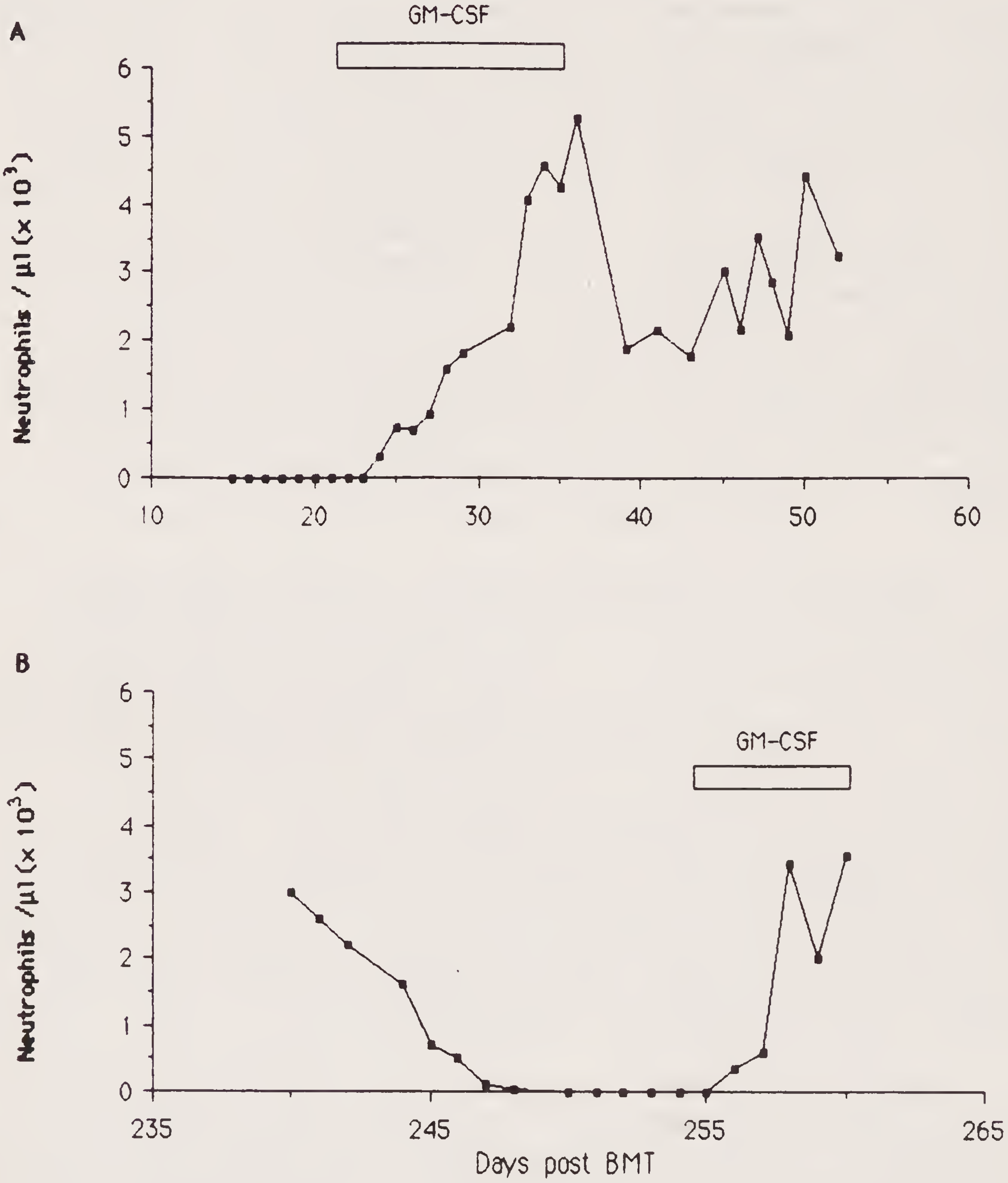
Non-hematopoietic tumor cell lines can also respond in some cases. This could be shown for breast cancer, osteosarcoma and some colon carcinoma cell lines (Dedhar et al., 1988). Any clinical trial designed to test GM-CSF has to take these results into consideration. However, at this point in time, it is clearly too early to draw any definite conclusions since these transformed cell lines may actually behave differently from the in vivo situation.

The study published by Antman et al. (1988) in patients who received GM-CSF after high dose chemotherapy for the treatment of osteosarcoma before and after chemotherapy describes an interesting observation. When the investigators compared their data in terms of remission rate, they found that patients who had received GM-CSF prior to chemotherapy, had a much higher complete remission rate than patients in the historical control group that did not receive the growth factor. The investigators may have unintentionally conducted an interesting experiment: GM-CSF given prior to chemotherapy might have put the malignant cells into cell cycle, and the subsequent chemotherapy may have encountered more cells in S-phase. This certainly needs further confirmation in clinical trials, but may open a new indication for the use of GM-CSF.

Bone marrow transplantation requires high dose chemotherapy sometimes in combination with radiation to eliminate the malignant clone, to suppress the immune system of the host and to create space for the transplanted marrow. It usually takes 2-3 weeks for the new bone marrow to engraft. Studies from Seattle and Duke University have shown that GM-CSF can accelerate engraftment after autologous marrow infusion (Brandt et al., 1988; Nemunaitis et al., 1988). On an average, leukocytes recovered 5-7 days earlier than in historical control patients with the same disease and preparative regimen. This also resulted in fewer febrile days, fewer days on antibiotics and overall lower morbidity and mortality.

There is another indication for the use of GM-CSF after marrow transplantation in which we are particularly interested. Occasionally patients have delayed engraftment and even 3 or 4 weeks after marrow infusion, there may be no signs of bone marrow engraftment. Conversely, there is a group of patients who, after allogeneic transplantation, may develop graft failure after initial stable engraftment (graft rejection). We have administered E. coli derived GM-CSF (10 µg/kg daily as a 30 min. infusion provided by Schering, Pointe Claire, Quebec) to these patients on compassionate grounds and found an increase in neutrophils within 24 hours of starting GM-CSF. In 6 of 7 evaluable patients a marked rise in neutrophil count was seen which was sustained in those patients who survived (Figure 1). There was no effect on platelet and reticulocyte counts.

Figure 1.



Effect of GM-CSF given in 2 patients after allogeneic bone marrow transplantation for (A) delayed engraftment and (B) graft failure.

Side effects were minor although we expected a high peak plasma concentration with the half hour infusion due to the fact that the material was from E. coli. One patient experienced abdominal cramps and diarrhea with each GM-CSF infusion. In another patient, we saw a macular rash. Of note was the fact that two patients developed hemorrhagic cystitis after two and four days of GM-CSF treatment and this condition resolved after discontinuation of the treatment. Any correlation to GM-CSF is speculative, particularly since both patients received cyclophosphamide during the preparative regimen which can cause hemorrhagic cystitis in a certain proportion of patients. However, the possibility that GM-CSF can cause a bleeding disorder should be considered. A recent report by Champlin et al. (1989) also mentioned bleeding during GM-CSF treatment in one patient. We did not see any worsening of graft versus host disease in patients who received GM-CSF for graft failure after allogeneic marrow transplantation.

Based on our observations, it is certainly reasonable to give GM-CSF to patients with delayed engraftment or graft failure. We have learned from these studies that it may be better to give GM-CSF earlier in the post transplant course before renal failure and other complications develop. Also, the dose might be better given over 4-6 hours to prevent high peak plasma concentrations and possible side effects related to that.

GM-CSF is not only capable of increasing numbers of neutrophils and monocytes/macrophages, but it also enhances several **functions** of these cells (Table 3):

Table 3 **GM-CSF Can Enhance Effector Cell Functions**

*Phagocytosis
*Chemotaxis
*Cytotoxicity
  - microbial
  - tumor cells
*Oxidative metabolism
*Antigen processing

We have tested GM-CSF to see if it can enhance the cytotoxicity of monocytes against certain tumor cell lines. GM-CSF alone can induce tumor cell killing in normal monocytes as well as in monocytes from marrow transplant patients after 18-24 hours incubation. This cytotoxicity is mediated through tumor necrosis factor (TNF) as shown by neutralization experiments with anti TNF antibody and use of specific TNF sensitive cell lines such as WEHI 164 (manuscript in preparation). Moreover, when monocytes were incubated for a few hours with γ-interferon, this **priming** effect increased the ability of monocytes to become cytotoxic after culture with GM-CSF. These in vitro observations form the basis for a novel adoptive immunotherapy using lymphokine activated monocytes (LAM) to eliminate residual disease, specifically leukemia after chemotherapy with and without bone marrow transplantation. Technically, large numbers of monocytes are removed from the patient by leukopheresis and are primed in vitro with γ-interferon before they are reinfused into the patient who then receives GM-CSF as a continuous infusion.

GM-CSF is only one hematopoietic growth factor that has become available recently in recombinant form. The future belongs to the combination of various lineage specific as well as multipotent cytokines. A first example is the use of IL-3 along with GM-CSF (Donahue et al., 1988) which not only allows us to lower the concentration of GM-CSF, but also stimulates megakaryocytopoiesis more effectively. There is no doubt that we have just entered a new era of treatment options for certain hematological disorders.

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Induction of Secondary Neoplasms by the Treatment of Malignant Disease: Lessons from Hodgkin's Disease

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Hodgkin's disease was among the first human malignancies shown to be curable even when widely metastatic. Sequential advances in pathologic classification, diagnostic evaluation, wide field irradiation and treatment using multiagent chemotherapy were made over about a century culminating in our ability to cure patients with even quite advanced disease. A disease which had a fatal outcome in more than three quarters of patients three decades ago now is expected to kill no more than 10 to 20% of those found to have it in 1989. These dramatic advances present clinicians and investigators with the challenge of recognizing and minimizing the toxic effects of the successful treatment, especially those which are permanent or life threatening. In particular, the induction of second neoplasms by potentially carcinogenic treatments must be anticipated, patterns of occurrence identified and treatments altered, if possible without reducing efficacy to reduce the number and range of these acquired malignancies. This brief review will summarize present observations relevant to the induction of second neoplasms by the treatment of Hodgkin's disease and conclude with a collation of what we have learned to date in this area.

Background

In North America Hodgkin's disease occurs with a bimodal incidence according to age with peaks between the ages of 20 to 30 and again after age 50. Thus, given the greater numbers of younger people in the general population the majority of cases are seen in young adults who have an otherwise long life expectancy and are at particular risk for late effects occurring even decades after treatment. The standard treatments, described in detail in Kaplan's classic monograph (1), are based either on wide field irradiation to multiple centri-axial lymph node groups or the use of multiagent chemotherapy or both. Because Hodgkin's disease is typically supradiaphragmatic the irradiation is usually delivered to a so-called "mantle" field which includes the lymph nodes of the neck, axillae and mediastinum, often extending down to include the upper para-aortic, celiac and splenic hilar lymph nodes and the spleen itself. Usual doses range from 3500 cGy to 4400 cGy given in 15 to 20 fractions over three to four weeks. The normal structures of the neck, thorax and upper abdomen are routinely exposed to these therapeutic doses or, if outside the fields of treatment, to variable amounts of scatter radiation.

The multiagent chemotherapy used for Hodgkin's disease has steadily evolved to the current era in which regimens containing seven or eight drugs are common (2,3). Most curative regimens include agents from several classes including alkylating agents such as mechlorethamine, cyclophosphamide, procarbazine or a nitrosourea, an anthracycline antibiotic such as doxorubicin, vinca alkyloids such as vinblastine or vincristine, corticosteroids such as prednisone and miscellaneous agents such as bleomycin or etoposide. Several of these, especially the alkylators and nitrosoureas are known carcinogens in both animal and human systems.

Certain non-neoplastic late or permanent side effects noted in patients treated successfully for Hodgkin's disease have recently been fully reviewed by Bookman and co-workers (4,5) and are useful to examine because the pattern of injury seen with these lesions is closely paralleled by the later development of solid tumors. These late effects and the relevant end organs are summarized in Table 1.

Table 1. Selected non-neoplastic late complications of the treatment of Hodgkin's disease and associated end organs at risk for second neoplasms.

Toxicity	End Organ at Risk
Pulmonary or pericardial fibrosis	Lung
Dental Caries	Oropharyngeal mucosa
Infertility or premature menopause	Uterine cervix, breasts
Hypothyroidism	Thyroid
Radiation fibrosis, any organ	Connective tissue Skin
Radiation enteritis	Gastrointestinal tract

The regional relation to fields of irradiation for both these non-neoplastic sequelae and as we shall see below, the solid tumors which develop later strongly suggest a major etiologic role for the irradiation used in the primary treatment.

Second Neoplasms

Contributing factors: time

One useful way to examine the issue of induction of second neoplasms after Hodgkin's disease is to determine the relation of time after treatment to the type of neoplasm seen and to measure what contribution to excess mortality over that present in the normal population is contributed by the Hodgkin's disease and what by other specific second neoplasms. During the first decade after diagnosis of Hodgkin's disease virtually all excess mortality is due to the Hodgkin's disease itself or the immediate toxicity, usually infectious, of the treatment used. However, after about ten years, although a small number of deaths from Hodgkin's disease continue to occur, the major contribution to excess mortality is from late complications of treatment (6). By about the fourteenth year after diagnosis all of the excess mortality is due to second neoplasms and virtually all of the second neoplasms are solid tumors (7).

The impact of time is even more striking when we examine the incidence of the first clearly recognized second neoplasm due to treatment of Hodgkin's disease: acute non-lymphocytic leukemia. Initial observations that the period of risk did not continue indefinitely (8) have been elegantly supported by the analysis of Blayney and coworkers (9) showing that the risk of developing secondary leukemia is sharply increased from the third to sixth years after treatment and falls to the background rate after ten to twelve years. Thus the risk of developing treatment induced leukemia does not seem to persist for a prolonged time. Rather, it peaks after three to six years and falls to negligible levels after a decade.

The findings relating time and incidence of second neoplasms after Hodgkin's disease can be concisely summarized. During the first decade after the disease excess mortality is from uncured Hodgkin's disease. During this same decade acute leukemia makes a small contribution to excess mortality. However, after that first decade neither Hodgkin's disease nor acute leukemia contributes substantially to excess mortality. Instead, after a decade second solid tumors make the major contribution to increased mortality and by the latter half of this decade account for all excess deaths.

Contributing factors: type of treatment and age

Additional useful information can be derived from analysis of the relation between the type of treatment and the type of second neoplasm. Several authors have now clearly documented the clearcut relationship between chemotherapy with regimens containing alkylating agents and the eventual occurrence of leukemia (5-8). Although irradiation by itself makes only a minor contribution to the risk of leukemia (1,5) its addition to chemotherapy which includes alkylating agents (5,6,8) nearly doubles the risk to approximately 4 to 5% at ten years. Thus, with regard to leukemia the major inducing factors are alkylating agents and, when combined with them, irradiation. The correlation of cumulative dose of alkylating agent with rising incidence of secondary leukemia (5) and the apparent very low incidence of leukemia after regimens which contain no or weak alkylators, even when combined with irradiation (10) are two additional observations strongly supportive of the hypothesis that acute leukemia is a direct consequence of the use of alkylating agents, added to by irradiation if combined modality therapy is used.

The evidence linking treatment and solid tumors is less clearcut. Because a wide variety of solid tumors have been seen and because many, such as lung and breast carcinoma, are among the most commonly encountered solid tumors in the general population obvious patterns of increased risk have not been identified. It is only in aggregate that the number of deaths from solid tumors clearly exceeds that of the normal population. However, certain patterns and possible increased incidence will be worthy of close attention in the future. These are summarized in Table 2 (4-7, 11-14).

Table 2. Observations linking treatment for Hodgkin's disease with subsequent solid tumors

Solid Tumor	Observation
Malignant lymphoma	Follow solid tumor incidence in time to occurrence and anatomic distribution
Lung carcinoma	Associated with thoracic irradiation with or without chemotherapy Smoking increases risk Usually small cell or adenocarcinoma types
Sarcoma	Usually in or at borders of radiation fields
Breast carcinoma	Associated with thoracic irradiation with or without chemotherapy Possible increase in bilaterality Possible increased occurrence in the young
Thyroid carcinoma	Associated with neck irradiation
Head and neck carcinoma	Associated with mouth and neck irradiation
Melanoma	Increased in patients with dysplastic nevus syndrome

Several of the secondary solid tumors, including lung, breast, head and neck, and thyroid carcinomas and soft tissue sarcomas are seen in organs within or near fields of irradiation. Unusual or restricted types, namely, diffuse histologies in the lymphomas, small cell and adenocarcinoma in the lung carcinomas, and bilateral disease in breast carcinoma are encountered at possibly increased frequency. This difference in the frequency of the various histologic subtypes indicates that these secondary neoplasms differ from those seen in the general population.

Age interacts in two ways. The sarcomas, thyroid and breast cancer seem to occur at younger ages than expected and are more likely to be seen in patients who were younger at the time of initial treatment. On the other hand, there is an overall increase in risk of second solid tumors as age at time of primary treatment increases. Finally certain tumors such as malignant lymphoma and melanoma may be occurring most often in those who received combined modality therapy hinting that the immunosuppressive impact of the treatment may be a contributing factor.

The long latency to second solid tumors, described above and quantified by Tucker and co-workers (7) complicates any observations about these malignancies. Relatively few prospective cohorts of patients have been followed for more than a decade. When such groups can be found certain institutionally chosen treatment programs which are no longer used such as adjuvant treatment with radioactive colloidal gold (7) or extensive MOPP (mechlorethamine, vincristine, procarbazine and prednisone) and wide field irradiation (6-9) may have been employed during the treatment era. The relevance of observations from such groups for patients being treated now can be questioned. Finally, the major limitation on all available observations is the small number of patients who have been followed off treatment more than fifteen to twenty years, a period of time which may be minimal in terms of second solid tumor induction. In this regard, Tuckers' actuarial prediction of a 17.6% overall second neoplasm and 13.2% second solid tumor risk at fifteen years is worrisome (7). Obviously, additional observations over longer periods of time of patients treated with different programs will be needed to unravel this complex story.

Conclusion

Patients cured of Hodgkin's disease are left with a long term increased risk of developing a second neoplasm. The risk of leukemia is most clearly related to the use of alkylating agents in the primary treatment but is augmented by irradiation. Overall, the impact of leukemia is minor and adds only a small number of excess deaths during the first decade of follow-up, a period when death from uncured Hodgkin's disease itself remains the greatest threat to the patient. Solid tumors, especially of lung, breast, head and neck, thyroid, skin (melanoma), connective tissue (sarcoma) and lymphoreticular tissue (lymphoma) assume major importance in the second decade of follow-up and contribute virtually all of the excess mortality beyond ten years. Although complex in etiology such solid tumors are most likely causally linked to the radiation therapy used for the Hodgkin's disease. Augmentation by direct cytotoxicity or immune compromise from chemotherapy is also probably playing a role. Longer follow-up of cohorts of patients treated with a variety of programs will be needed to provide a clearer picture of the nature and duration of the induction of second neoplasms in patients successfully cured of Hodgkin's disease.

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OVERVIEW OF ADJUVANT RADIOTHERAPY FOR BREAST CANCER

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INTRODUCTION

A companion pair of papers has been published recently in which mortality results from all available mature randomized trials of adjuvant radiotherapy were updated and analyzed collectively (Cuzick et al, 1987a,b). These overviews were based on individual patient data for over 10,000 patients of which more than 6000 had died. Brief details of the individual trials are shown in Table 1. As shown in Figures 1 and 2, no effect of radiotherapy could be found in the first ten years of follow up in the two types of pure radiotherapy trials – radical mastectomy with or without radiotherapy, and simple mastectomy with or without radiotherapy. There was no indication of heterogeneity between different trials. In trials of simple mastectomy with radiotherapy vs radical mastectomy without radiotherapy, a slightly worse prognosis was observed in the irradiated patients ($P = 0.05$). See Figure 3. This was due to poor survival in one trial (Southeast Scotland) in the first 5 years of follow up and appears to be a chance finding.

However, the data suggested rather strongly ($P < 0.001$) that at least some forms of radiotherapy had a deleterious effect on long–term survival as measured by subsequent survival in those patients who survived at least ten years. This result was based primarily on the earliest generation of trials in which radiotherapy was a randomised option after all patients had received a radical mastectomy (Figure 4), but was also supported by the Kings/CRC trial of simple mastectomy with or without radiotherapy (hazard ratio after 10 years = 1.50, 95% confidence interval (1.08, 2.09, $p < 0.05$). The other trials of simple mastectomy with or without radiotherapy were not sufficiently mature to yield very much information about survival after 10 years. However, from the information available, there was no suggestion of heterogeneity

TABLE 1. Details of radiotherapy regimens in trials under review

Trial	Yr of onset	Type of therapy	Dose (Gy)	No. of fractions	Duration (days)	Biologic dose (rets)
Radical mastectomy ± XRT						
Manchester Q	1949	250 kV	35.0-40.0	15	21	1438-1693
Manchester P	1952	250 kV	32.5-42.5	15	21	1335-1746
Oslo I	1964	200 kV	18.0-36.0	20	28	681-1362
Oslo II	1968	^{60}Co	50.0	20	28	1689
Heidelberg	1969	^{60}Co	54.0	30	42	1905
Stockholm	1971	Nodal, ^{60}Co	45.0	25	35	1406†
Simple mastectomy ± XRT						
Manchester Regional	1970	300 kV	37.0	15	21	1493
		or				
		Nodal, 4 meV	40.0	15	21	1464 (1211‡)
			30.0	15	21	
CRC	1970	Ortho	32.5–46	—	21-42	1335-1483
		or				
		meV	35.7-50.6	—	21-42	1306-1453
NSABP-B04	1971	meV	40-50	25	35	1224-1530
Edinburgh I	1974	4 meV	42.5	10	28	1661
Simple mastectomy + XRT vs Radical Mastectomy − XRT						
Copenhagen	1951	Nodal, 400 kV	42.0	18	21	1592 (1652*)
Southeast Scotland	1964	2 meV	42.5	10	28	1695
Manchester Regional	1970	300 kV	37.0	15	21	1493
		or				
		Nodal, 4 meV	40.0	15	21	1464 (1211‡)
			30.0	15	21	
NSABP-B04	1971	meV (node-negative)	40-50	25	35	1224-1530
		meV (node-positive)	50-60	25	35	1531-1837

*Chest wall, 250 kV.

†Chest wall, 10–15 meV.

‡Chest wall, 300 kV.

between trials. Overall the death rate after 10 years in 10−year survivors was estimated to be 35% higher (95% confidence interval 15%−58%) in those who had been randomized to receive radiotherapy.

These results have prompted further questions which were not addressed in the original study. These are of two types (1) limitations in the data base, and (2) limitations in the analysis. These are briefly addressed below.

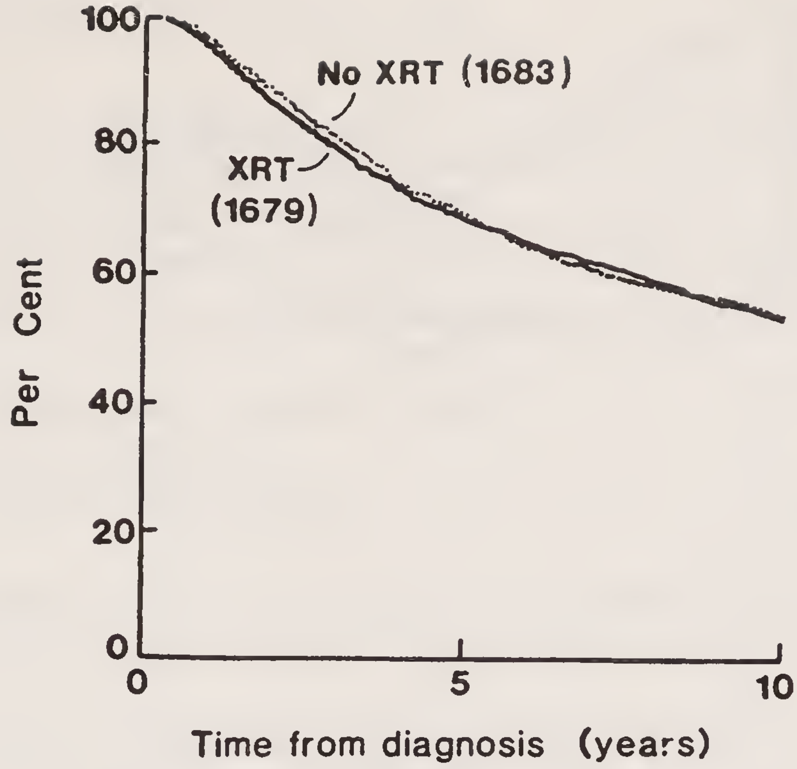

Figure 1. Combined 10—yr survival according to allocated treatment for trials employing a simple mastectomy. Curves do not include material from NSABP—B04 trial. Nos. in parentheses are total patients at risk in each arm (excluding NSABP).

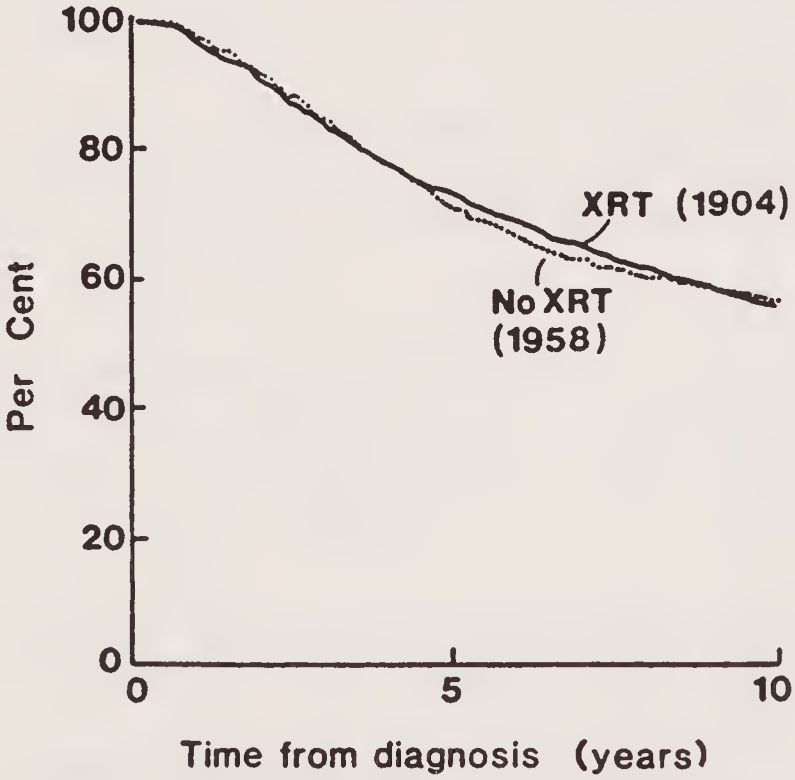

Figure 2. Combined 10—yr survival according to allocated treatment for all trials employing a radical mastectomy. Nos. in parentheses are total patients at risk in each arm.

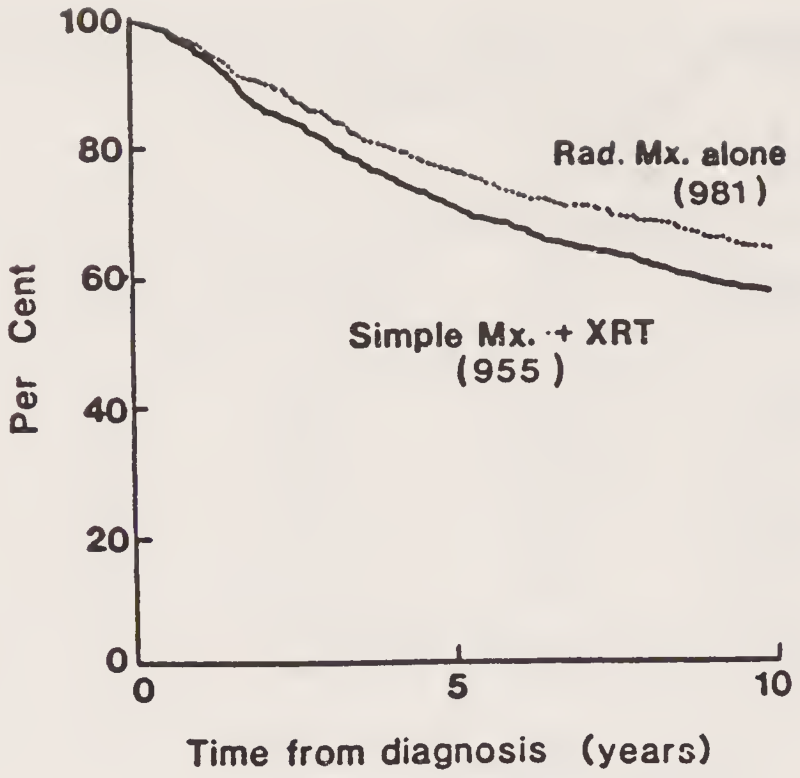

Figure 3. Combined 10−yr survival curves in trials comparing radical mastectomy alone vs simple mastectomy with radiotherapy. Survival was marginally better in the nonirradiated arm (P = 0.05). Nos. in parentheses are total patients at risk in that arm (excluding NSABP). Curves do not include data from NSABP−B04 trial although all calculations do.

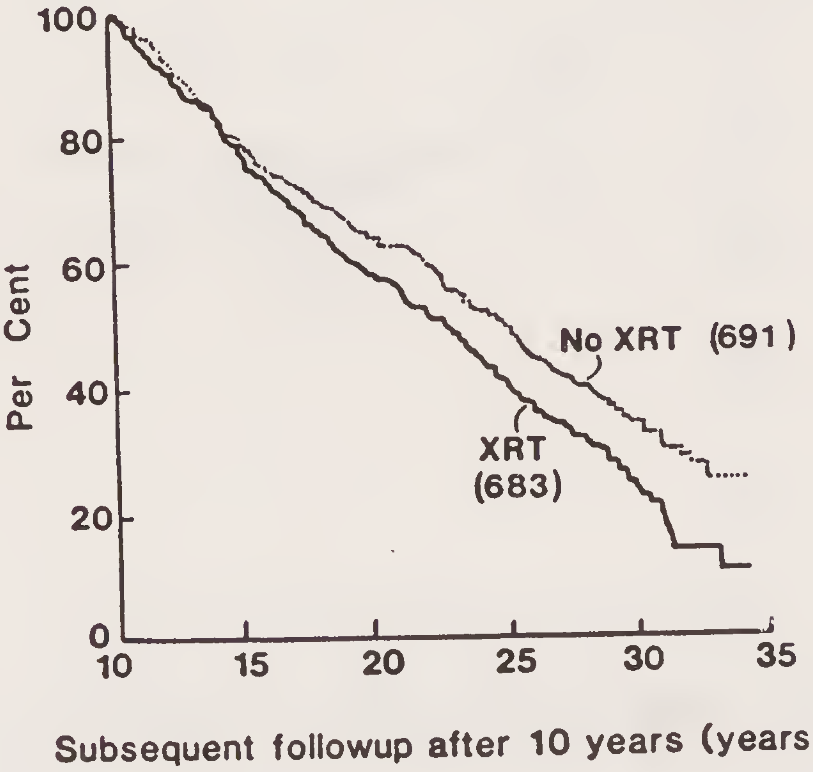

Figure 4. Subsequent survival in patients surviving 10 yrs who were in a trial employing radical mastectomy. There is a significant difference favouring patients treated by radical mastectomy alone (P = 0.002). Nos. in parentheses are total patients at risk in each arm.

LIMITATIONS IN THE DATA BASE

Because of the large amount of effort necessary to extract data from very old trials, the data collected were kept to a minimum. We were interested in long term mortality, so that it was essential to record the vital status and date of death of each individual patient. The essential core data collected on all patients consisted of:

(i) randomised treatment option
(ii) age at diagnosis
(iii) clinical nodal status.

The results of the overview have prompted a number of further questions including:

(i) Which specific cause(s) of mortality are elevated?
In particular are leukaemia, other cancers, or heart disease mortality elevated.

(ii) The relation of excess mortality to type of radiotherapy given.
The scattering profile and organ doses vary considerably according to whether orthovoltage or megavoltage machinery is used and also depends on the particular fields irradiated and doses given.

(iii) Laterality of primary tumour. This will affect doses to other tissues, especially the heart, and an examination of heart disease mortality in relation to which breast was irradiated may be informative.

(iv) Actual treatment given, not just allocated treatment.
Few patients did not receive the allocated initial treatment, but many had radiotherapy for local recurrence and some of these will have survived more than 10 years.

In order to answer these questions we plan to collect the following additional information on each individual patient who survived 10 years or more:

(i) Whether breast cancer was present at death.

(ii) Cause of death with special attention to the following major groups:

a) breast cancer

  - b) other cancer
  - c) cardiac
  - d) other vascular

(iii) Type of radiotherapy given, especially kilovoltage vs megavoltage X-rays.

(iv) Actual radiotherapy given to primary site – dose and fields both at presentation and for relapses in the first 10 years.

(v) Laterality of original primary tumour.

(vi) Radiotherapy given for contralateral disease.

Results from a number of the participating trials will be published soon and they do seem to point towards an excess of heart disease in irradiated patients. Haybittle et al (1989) found an excess of cardiac related mortality and also an excess of other cancers. The excess of heart disease appeared to be related to irradiation of tumours in the left breast and also possibly to orthovoltage irradiation which is now largely superseded by megavoltage machines. The fundings reported in this study need to be compared with those in other trials. We hope this more detailed overview will help to answer questions about the long term effects of radiotherapy, which are particularly relevant now, because of a shift in primary treatment towards breast conservation with its associated increase in the use of radiotherapy.

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MEASURING QUALITY OF LIFE

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INTRODUCTION

One hundred years ago, before the advent of modern cancer therapy, the quality of life of a patient with cancer was affected almost entirely by the disease itself. Fifty years ago, radiation therapy and surgery were the major modalities available for the treatment of cancer. In this era, the quality of life of a patient with cancer was affected not only by the disease but also by the effects of treatment. Today, not only are chemotherapy and biologic therapy available in addition to the other two modalities, but chemotherapy is becoming ever more complex with the use of multiple-agent, non-crossreacting combinations and dose-intensive regimens. Biologic therapy is also accompanied by significant side effects. Thus, in the modern era of cancer therapy it is necessary to take into account not only the disease-related effects but also the treatment-related effects on quality of life. The relationships between these effects and the ultimate benefit derived by the patient form a complex, interacting set of pofactors that need to be assessed in order to make decisions in the best interest of the patient.

The assessment of quality of life and the expression of the results in quantitative terms can provide a rational basis for description, evaluation and decision-making. Although each individual physician already performs a qualitative assessment of quality of life in a conscious or subconscious manner when dealing with individual patients, there is a need to be able to express this assessment more formally in quantitative terms so that the information can be communicated to others and used for comparative purposes when attempting to describe the effects of the disease and its treatment and attempting to make appropriate decisions in groups of patients or populations.

An appropriate application of the quantitative assessment of quality of life is to combine the results with the results of the quantitative assessment of the quantity of life, ie. survival. Together these assessments provide an estimate of quality adjusted life years of survival (QALYS). Thus, a single numerical expression is available for analyzing cost-utility (Drummond et al 1987), and this can be used for intergroup comparisons within populations of patients with cancer or for interpopulation comparisons of patients having cancer with other diseases. Without adjusting for QALYS, it is not possible to resolve the dilemma of how to compare patients who experience an increase in quantity of life which may have decreased quality with patients who experience no change in quantity or quality of life as a result of treatment.

Can quality of life be measured? This article attempts to show that it can be done and is being done. Although refinement in present methodology is still required to develop ideal assessment methods, several of the available self-assessment questionnaires have sufficient reliability for use both in clinical trials and in individual patients.

DEFINING QUALITY OF LIFE FOR THE DEVELOPMENT OF ASSESSMENT TOOLS

In order to be able to develop meaningful assessment tools, it is necessary first to define quality of life. Till, McNeill and Bush (1984) have provided a useful definition, since it suggests the kinds of questions that need to be answered in a quality of life assessment. They state that quality of life "is a global concept that.......includes psychologic and social functioning as well as physical functioning and incorporates positive aspects of well-being as well as negative aspects of disease or infirmity". This definition suggests that there are at least three dimensions to quality of life that need to be considered. These are the physical, psychological and social dimensions. Other workers have suggested that additional dimensions are also of importance, eg. disease symptoms, treatment side-effects, sexuality and body image, satisfaction with medical care and somatic discomfort (Aaronson et al, 1987). Having identified the relevant dimensions, the next step in devising quality of life assessments is to develop questions which assess appropriate constructs within each dimension. Several of the most commonly used quality of life measures incorporate three or more of the dimensions given above. It would appear that the most fundamental dimensions are the physical, psychological and social ones and most other dimensions are related to these three fundamental ones.

WHO SHOULD ASSESS QUALITY OF LIFE?

In attempting to define quality of life, authors have used phrases such as "the degree to which an individual succeeds in accomplishing his desires" (Gerson, 1976), "the extent to which a persons hopes and ambitions are matched or fulfilled by experience" (Calman, 1984), and "quality is a comparative property...a comparison of the qualities this patient now has with the qualities deemed by this patient to be normative and desirable..." (Keyserlingk, 1979). In these definitions, the use of the words "degree", "extent", and "comparison" implies that there is a normative standard in the mind of the subject when responding to questions about his quality of life. Who, then, should be asked to assess the quality of life of a patient? If the criterion normative standard is the one held by the patient, then the answer is that only the patient can provide an assessment of his own quality of life. If an observer, eg. health care worker, assesses the quality of life of a patient, the answer will be based upon the observers normative standard and not the normative standard of the patient. Similarly, any other surrogate assessment suffers from the same deficiency. Therefore, only the patient can provide a meaningful response, since the patient will be comparing his present perceived state of health with his own present, perceived normative standard. In practice, studies have shown discrepancies between a patients and an observers perceptions of the patients quality of life (Padilla et al, 1983).

WHY SHOULD QUALITY OF LIFE BE MEASURED?

At the beginning of this paper, the statement is made that quality of life needs to be measured because of the need to know the overall effect of the disease and its treatment on the patient. Within this global reason, there are specific reasons why measuring quality of life may be useful.

In the context of clinical trials, measuring quality of life may help to provide a choice between treatments that have equivalent biological effects. For example, if the length of survival in two treatment arms of a clinical trial is not significantly different, the decision as to which treatment is preferable may rest upon the outcome of the quality of life assessment.

Both within clinical trials and outside of them, measuring quality of life provides an opportunity for earlier detection of morbidity since a systematic enquiry is likely to uncover disease or treatment effects as soon as they are perceived by the patient. Thus, measuring the quality of life can provide a more accurate description of the burden imposed by the disease and its treatment. Furthermore, knowing about quality of life can provide data which can form the basis of rational decision-making about further interventions for either the group or the individual. For example, the detection of a consistent pattern of morbidity could suggest a strategy for preventing the morbidity or ameliorating it at an earlier stage of development.

Another benefit of quality of life measures is to provide data for stratifying patients into more homogeneous groups of subjects which may, in turn, be entered into clinical trials of particular interventions designed to improve quality of life. In this context, performance activity scales (Karnofsky and Burchenal, 1949; Zubrod et al, 1960) frequently are used to stratify patients according to physical function. Further stratification based on psychological and social functioning would further increase the homogeneity of patient populations.

Finally, when psychological or social interventions are being tested in clinical trials, selection by eligibility criteria based on psychologic or social functioning would provide more homogeneous groups of patients for study.

HOW SHOULD QUALITY OF LIFE BE MEASURED?

The investigator may use either the interview method or the self-report method for obtaining the desired data. There are several advantages to using the self-report questionnaire approach. Firstly, a standardized format with the same questions being administered to all the patients in the group being studied ensures that responses are obtained to the same questions throughout the group. Self-report questionnaires can be constructed in such a way that they have both reliability (test-retest stability, internal consistency, and inter-institutional or inter-rater reproducibility) and validity (content, construct, concurrent, convergent, and predictive). Unfortunately, there is no criterion (gold standard) to which quality of life measures can be compared. However, criterion validity can be approached if the questionnaire is used repeatedly in different situations, and is shown to give meaningful information.

Quality of life measures should have been constructed for and validated in appropriate samples of patients, eg. if one wishes to measure quality of life in patients with cancer then the questionnaire to be used should have been developed in such patients. The use of a questionnaire developed in another population of patients, eg. psychiatric illness, college graduates etc., will have little meaning in patients with cancer.

Other desirable characteristics of quality of life measures include:

1. Questionnaires should be written in a language which is understandable to individuals with little education.

2. A questionnaire should be sensitive to changes resulting from the disease, it's treatment or co-morbid conditions.

3. A questionnaire should be able to distinguish between physical, emotional and social dimensions and should also be able to distinguish between the effects of the disease and the treatment.

4. A questionnaire should be feasible, ie. reasonably brief so that it can be administered repeatedly to very ill patients, be nonoffensive to patients, and be inexpensive to administer, score, and analyze.

5. Finally, a questionnaire should provide a basis for decision-making for future intervention.

In choosing which questionnaire would be most suitable for the patients to be studied, the above items can be used as a check list which will assist in coming to a decision.

A survey of currently available self-assessment questionnaires indicates that a number meet some, if not all, of the above requirements. These include the QL Index developed by Spitzer et al (1981), The Functional Living Index - Cancer developed by Schipper et al (1984), two breast cancer questionnaires - one developed by Selby et al (1984), and the other by Levine et al (1988), and the Quality of Life Questionnaire being developed by Aaronson et al (1987) for the Quality of Life Study Group of the European Organization For Research On Treatment Of Cancer (EORTC). This latter questionnaire is designed as a "core" questionnaire which would be applicable to most patients with cancer. In other words, it asks a number of general questions which would have meaning in most patients with cancer. However, there are many specific situations in which a core questionnaire would not be able to provide the appropriate data. For more specific situations, be they disease-specific, treatment-specific or situation-specific, separate modular questionnaires can be developed and added as appropriate. Thus, in patients with lung cancer it is possible to use the core questionnaire and add a specific module relating to symptoms of lung cancer and their effects on quality of life. This appears to be a rationale approach that is likely to be useful.

CONCLUSION

At the present time, no single questionnaire exists which would be the perfect questionnaire for all patients with cancer. Many of the questionnaires that have been proposed have not been psychometrically validated in patients with cancer or have not been validated adequately. However, some questionnaires do have desirable attributes and a list of these attributes has been provided. These can be used a check-list in attempting to choose the most appropriate questionnaire for the population of patients to be studied.

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CHEMOTHERAPY SCHEDULES: IMPACT ON TREATMENT OUTCOMES AND QUALITY OF LIFE

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INTRODUCTION

A wide variety of factors have influenced the development of cancer chemotherapy schedules. At differing times during the evolution of cancer chemotherapy, the influence of pharmacological considerations (pharmacokinetic and drug distribution data), of knowledge of tumour biology (particularly information on tumour cell cycle kinetics, tumour cell heterogeneity and causes of drug resistance) and clinical research (observation of dose response relationships and of toxic effects to normal tissues) have all contributed to the current drug schedules in common clinical use. The importance of experimental chemotherapy in transplanted animal tumors must also be acknowledged, with some drug combinations and schedules being shown to have supra-additive or synergistic antitumor effects. In addition in vitro studies of drug interactions in cell culture have in some cases contributed to new drug combinations and schedules being investigated in vivo.

The duration of chemotherapy programs has been influenced by additional considerations including the goals of treatment, the tumor response and its usual duration, patient tolerance and latterly by incorporating measurement of quality of life with other endpoints in overviewing treatment outcomes. Together these varied factors have, contributed to the diverse chemotherapy schedules which characterize contemporary cancer chemotherapy.

In this presentation two breast cancer chemotherapy studies will be reviewed. The first explores the scheduling of adjuvant chemotherapy in operable breast cancer patients by examining the antitumor effects of retreating patients, whose tumors recur, with the chemotherapy which they previously received as an adjuvant treatment. The second trial investigates the duration of chemotherapy in advanced breast cancer patients, and compares a policy of continuing treatment until the tumor relapses versus a policy of interrupting treatment after 3 cycles in patients whose tumor has responded to treatment, and reintroducing this chemotherapy at the time of disease progression.

PATIENTS

Patients in the first study were entered in the adjuvant breast cancer trials conducted by the Ludwig Breast Cancer Trials Group (LBCT, 1984, 1985, 1988). In 1982, a protocol was launched by this group to investigate the antitumor effects of retreating patients in LBCT trials who relapsed following adjuvant therapy with the same treatment they had received as adjuvant following mastectomy. This protocol restricted entry to patients with first relapse of breast cancer at least six months after cessation of adjuvant therapy. The protocol was not adopted universally by participating trial centres, and even in those centres entering patients on this protocol, many patients who relapsed were not retreated with the 'failed adjuvant'. To date information about response to retreatment is available for only 67 patients.

The patients in the second study were entered in the Australian New Zealand Breast Cancer Trial group of the Clinical Oncological Society of Australia (COSA) trial, which investigated different durations of initial chemotherapy for advanced breast cancer (Coates et al., 1985). The majority of patients entered in this trial had previously received endocrine therapy for metastatic disease (56%), but none had received cytotoxic chemotherapy, except as adjuvant. Patients were randomly assigned in a two by two factorial design to continuous or intermittent therapy with doxorubicin plus cyclophosphamide or with cyclophosphamide, methotrexate, fluorouracil and prednisone. If disease did not progress in the patients receiving intermittent therapy, their treatment was stopped wafter three cycles; if their disease later progressed, the same treatment was then given for three more cycles. This sequence was repeated until disease progressed during treatment. Patients were asked to complete quality of life assessments every 9 weeks. These were five linear analogue self assessment scales measuring physical well being, mood, pain, nausea and vomiting, and appetite, and a single uniscale summarizing overall quality of life.

RESULTS

67 patients with first relapse of breast cancer more than 6 months after completing adjuvant therapy after mastectomy were retreated with their 'failed' adjuvant treatment. 32 had received only a single perioperative course of intravenous CMF (Ludwig Breast Cancer Trials Group, 1988), and 35 had completed 6 or 12 monthly cycles of conventional CMF ± prednisone 7.5 mg/ daily ± tamoxifen 20 mg/daily (Ludwig Breast Cancer Trials Group, 19). 47 of these patients were premenopausal at the time of mastectomy and in 18, the treatment free interval was 6 - 12 months (interval between last adjuvant treatment and time of retreatment). Table 1 shows the outcome of retreatment. Overall there was a 52% tumour response rate, and in patients with only soft tissue recurrence the tumour response rate was 72%, with 47% having a complete response.

In the second trial, 305 patients are included in the analysis of treatment outcome. As of March 1988, 298 patients had died, and the study data are thus very mature. Figure 1 shows the time to first treatment failure of women receiving continuous chemotherapy compared to those on the intermittent therapies. The time to the first treatment failure was significantly shorter in the intermittent therapy arms. Figure 2 shows the survival according to these treatment groups. Survival did not differ significantly among the four treatment groups ($p = 0.30$ by log-rank test).

Changes in quality of life scores during the first three cycles of chemotherapy are shown in Figure 3. No difference was found between patients receiving continuous treatment and those receiving intermittent treatment. Analyses of changes in quality of life score after the continuous and intermittent treatments diverge is based on 66% of the potential number of patients. All endpoints favoured continuous therapy (Figure 4).

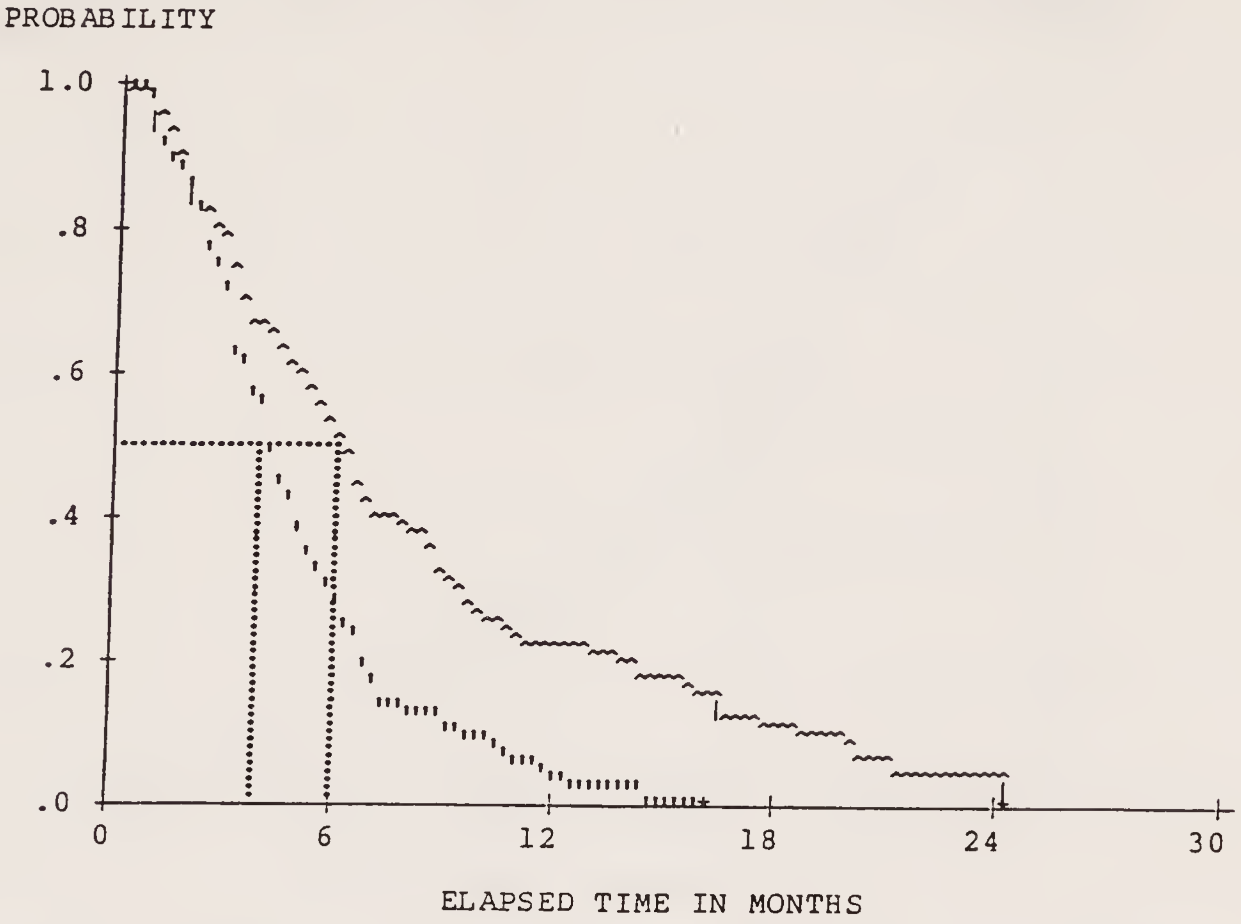
TABLE 1. Retreatment with CMF ± P ± Tam of women with relapsed breast cancer following completion of the same treatment given as adjuvant after mastectomy

Patients	Number	% Response to Retreatment	
		CR	PR
Single cycle i.v. CMF as adjuvant	32	37	16
6 or 12 cycles CMF ± P ± Tam as adjuvant	35	20	31
Total	67	28	24
Dominant metastatic site at time of retreatment			
soft tissue	36	47	25
bone ± soft tissue	10	0	20
visceral ± other	21	9	24

DISCUSSION

The results of the first study indicate that tumour relapse which is detected more then six months after completion of adjuvant chemotherapy in operable breast cancer patients does not necessarily imply that the relapsed tumour will be resistant to the drug used earlier as adjuvant therapy. This observation which confirms the report of other investigators (Valagussa et al., 1986), has prompted the International Breast Cancer Study Group to commence a large scale randomised trial in which the effects of reintroduction of adjuvant treatment after treatment free periods but while patients are disease free will be assessed by comparison with 3 and 6 monthly cycles of CMF. The possible explanation for the observed results is that some micrometastases in women with operable breast cancer may be temporarily insensitive to adjuvant treatment given during the first few months either because of being

Figure 1: Time to first treatment failure of patients receiving continuous or intermittent treatment.



Number at risk

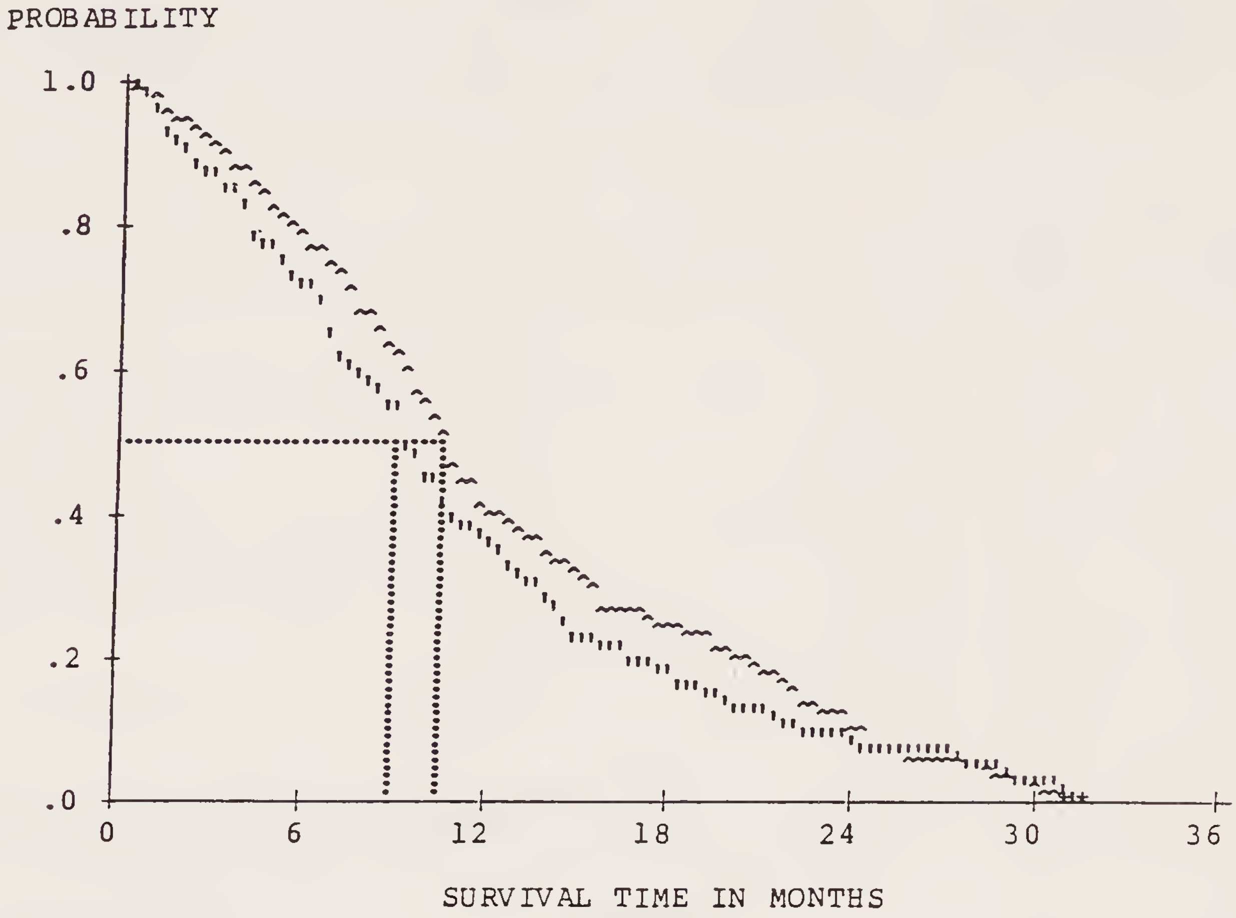
〰	154	65	17	8	1	0
''''	150	36	4	0		

Code	Curve	Median
〰	CONT	6.0
'''''''	INTER	4.0

out of the mitotic cycle (and thus 'resistant' to cycle active chemotherapy), or because of epigenetic changes contributing to relative drug resistance. One further may be reduced therapeutic approach which might improve the results of standard duration adjuvant chemotherapy would be to incorporate into treatment a drugs or growth factor which recruits micrometastases into the proliferative cell cycle. One concern with such a strategy is that disease free intervals in some patients because not all tumour cells recruited into cycle will be sensitive to the adjuvant chemotherapy.

The results of the second trial are also counter intuitive, in that the quality of life, response to

Figure 2: Survival of Patients receiving continuous or intermittent treatment.



Number at risk

〰	154	117	60	35	14	2	0
''''	150	107	54	27	12	3	0

Code	Curve	Median
〰	AC-CONT	10.3
'''''''	AC-INTER	9.0

treatment, the time to ultimate treatment failure and trend in survival duration all favoured continuous therapy. It might be thought that the lower quality of life found in patients receiving intermittent chemotherapy reflected increased patient anxiety during periods without chemotherapy. However the changes in quality of life scores were found to be significant independent predictors of the duration of subsequent survival. This finding provides a link with objective endpoints, and suggests instead that quality of life scores reflected the status of the metastatic disease.

The conclusions of this latter trial may not be applicable to chemotherapy administered at a different stage of disease or to other types or durations of intermittent chemotherapy. Nevertheless it is clearly appropriate to incorporate quality of life endpoints in future trials of chemotherapy in advanced breast cancer patients.

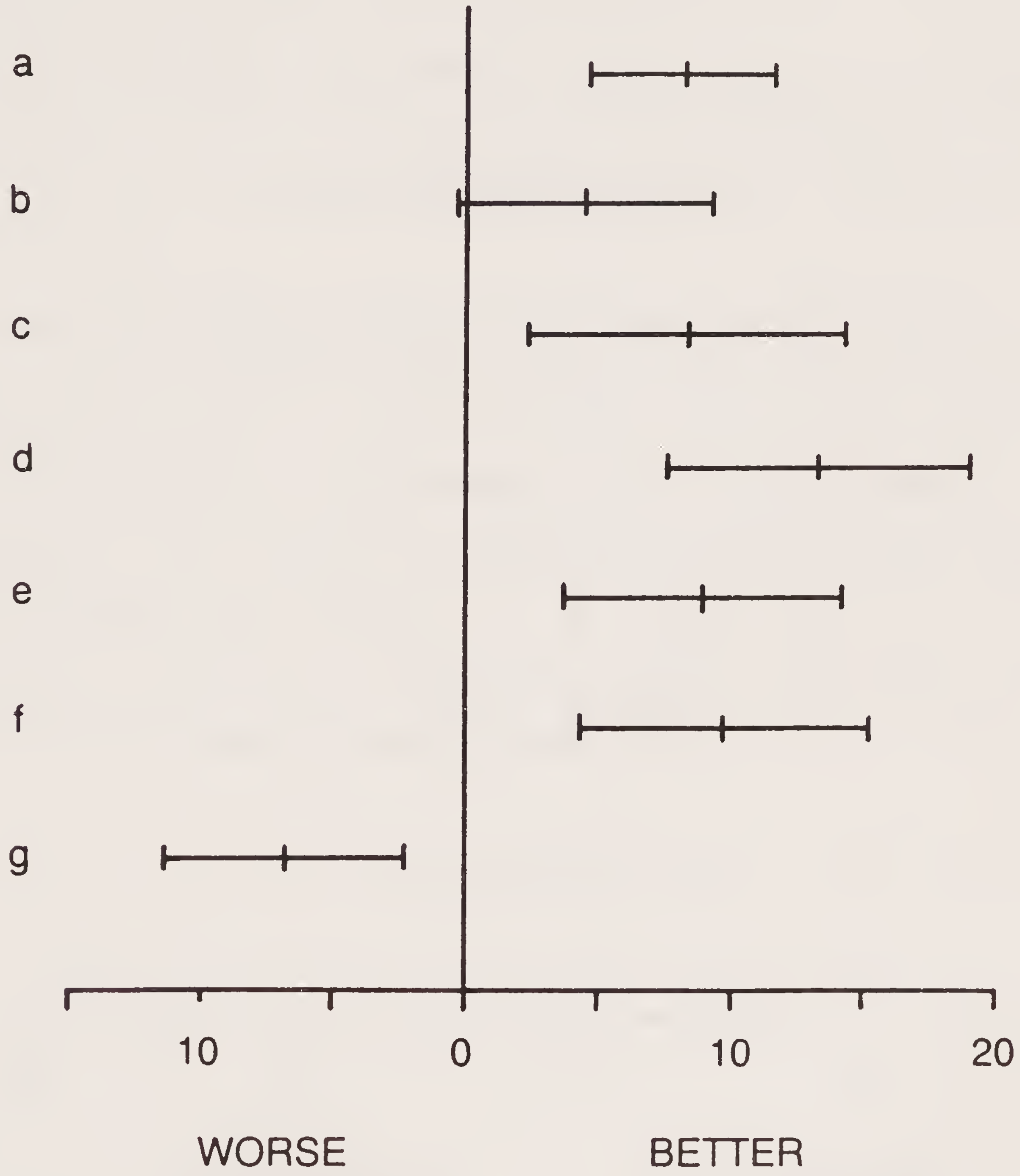


Figure 3. Changes in Quality-of-Life Scores during the First Three Cycles (All Groups Received Therapy).

The symbol a denotes the quality-of-life index, b the uniscale score, c the score for physical well-being, d for pain, e for mood, f for appetite, and g for nausea and vomiting.

Bars show 95 percent confidence intervals with means. The scale is expressed as a percentage of the possible range, corresponding to millimeters for linear-analogue scores.

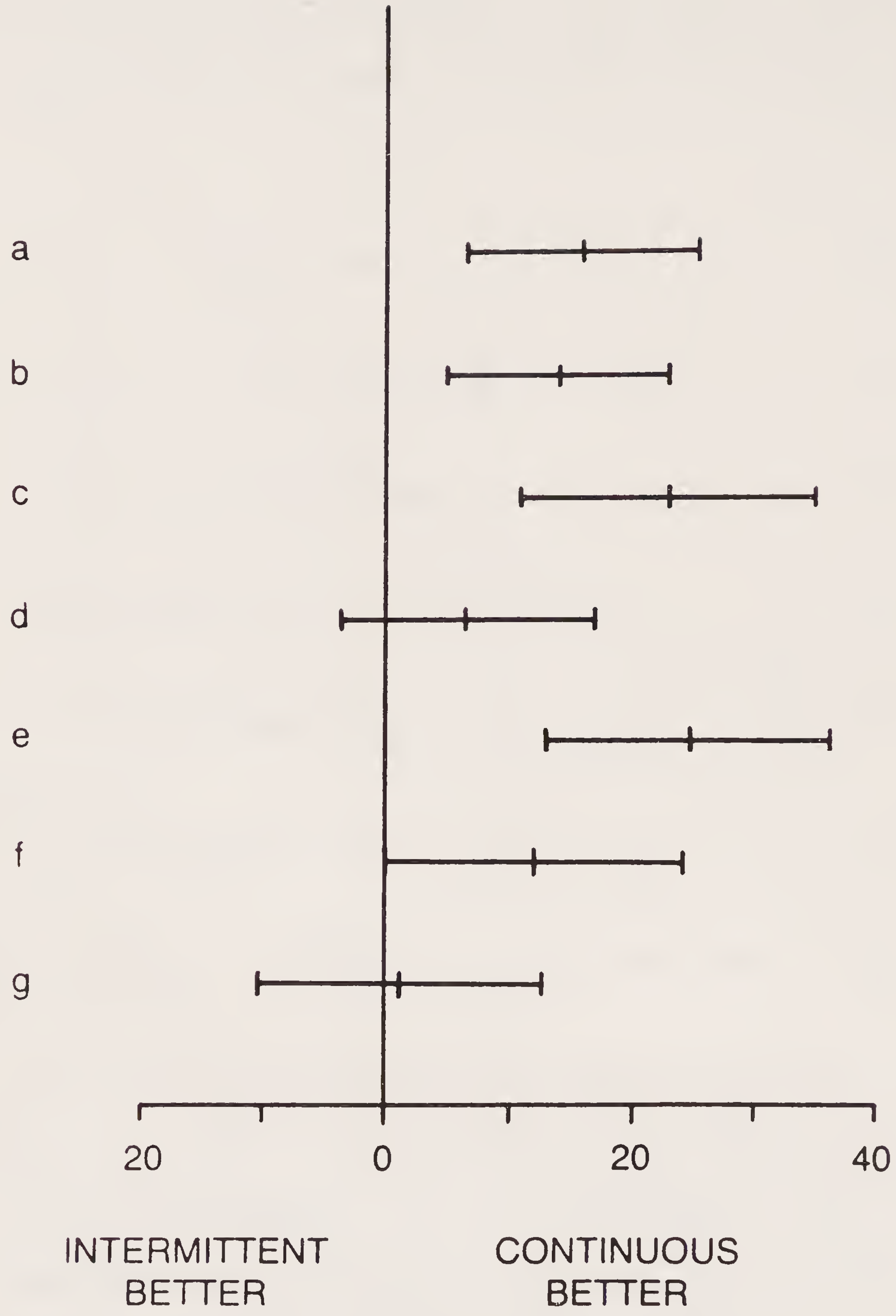


Figure 4. Changes in Quality-of-Life Scores after Intermittent Diverged from Continuous Treatment and before Disease Progression.

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COMMENTS ON FUTURE DEVELOPMENTS OF CANCER THERAPY. TAKING INTO CONSIDERATION TUMOR BIOLOGY AND KINETICS. PART II

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INTRODUCTION

The second volume of this Symposium proceedings focuses on issues relating to cancer therapy likely to gain a major momentum in the near future. Many treatment modalities and issues discussed here, such as biological response modifiers or high dose chemotherapy with autologous bone marrow transplantation, are newly introduced therapies. The intensity of their research, and the speed at which the new data is accrued, predicts that if the rate of progress continues, they may significantly improve the curability of human cancer. This is true, as well, of the ares of research discussed in the first volume, such as genetic manipulation, growth factors and differentiation, etc.

High dose intensity therapies may also result in a more significant impact on cure rates, but in parallel, their side effects, including long-term organ toxicity and treatment induced mortality or increased incidence of second malignancies, will also inevitably increase. Hence, a very thorough and unbiased assessment of their overall impact on the natural history of cancer remains a main challenge for the future investigators. In the closing remarks, several final comments regarding key issues discussed in this volume will be made.

FUTURE COMMENTS REGARDING BIOLOGICAL RESPONSE MODIFIERS (BRM).

The areas of clinical applications in which BMR will likely be first employed in routine future treatment include the utilization of cytokines such as G-CSF or GM-CSF, as support therapy for virtually all conditions where bone marrow suppression is the main concern. Presently available data of their early trials as a rescue after dose intensive chemotherapy indicate a significant reduction in duration of granulocyte suppression, infection, fever and hospitalization. Also, a more rapid engraftment of autologous bone marrow transplantations is seen and their use in conditions such as AIDS and radiation accidents has already been implemented.

The following points regarding the role of support cytokines will need clarification:

1.) To what degree will cytokines be of assistance to the average clinician in the routine management of malignancies (as opposed to their role as a research tool only);
2.) Concerning their role vis-a-vis autologous bone marrow transplantations (ABMT) - is their bone marrow rescue effect sufficient enough to permit their replacing ABMT, or would they be needed primarily as adjunct to ABMT?

3.) Do Cytokines have a direct stimulatory activity against solid tumor stem cells? If the support cytokines indeed are proven to stimulate solid tumor cell lines, will the result of such an action be, on the one extreme, beneficial as in the enhancement of the chemothotherapeutic effect by increasing the tumors' growth fraction. Or on the other extreme, will there be a deleterious effect as a result of an uncontrolled direct stimulation of cell division, resulting in tumor progression?

Other cytokines, including interferones and interleukins, show promise in the clinical setting, as seen from the preliminary data of early trials of interferon therapy in hairy cell leukemia, or effects of IL-2 in renal cell carcinoma or melanoma. Their impact looks very promising, particularly for tumors with no responses to conventionally applied chemotherapeutic agents. Cytokines may represent a resurgence of truly non-cross resistant therapeutic modalities. Also, the fact that most BRM are immune modulators suggests the possibility that responsive tumors may be amenable to other types of immune therapy such as monoclonal antibodies. This observation may also be of significance in the field of immunodiagnosis, either when monitoring for signs of early metastases of established resected tumors, or importantly, in diagnostic or screening efforts applied to high risk populations.

COMMENTS ON RADIOBIOLOGY

Several aspects of radiation research deserve comment. As discussed by most authors of this section, hypoxia remains the main cause for radioresistance, and manipulation of oxygen delivery either with by now well known radiosensitizers such as misonidazole, or alternatively, by manipulation of blood supply and tumor perfusion utilizing radiosensitizing effect of agents activated under hypoxia, are all research projects underway, likely to bring significant improvements. Other aspects of radiation utilization imply its ongoing combination with chemotherapy in which both modalities may benefit. In this interaction, chemotherapy sensitization of radiated tissues improves the radiation effects, and radiation debulking assists in the overall chemotherapy cell kill, both actions serving as an excellent example of benefits of combined modality approach. In this interaction, several issues such as optimum coordination of radiation - chemotherapy timing, need for altered dosing of either of the two modalities if applied concurrently, excess of organ toxicities as a result of combined treatment, as well as testing of new agents acting as potential radiosensitizers, etc., will all have to be researched in more detail.

Hyperfractionation and exploring such modalities as therapy with pi-meson particles, are additional steps aimed at improvement of the therapeutic ratio of radiotherapy. Lastly, application of techniques such as direct vizualization of cell division and other cellular processess, as described by Kallman in this section, may significantly improve the insight into the cell cycle, cell division, and overall tumor kinetics of irradiated or otherway treated cells, assisting thus greatly with understanding of tumor biology and application of new therapeutic approaches.

COMMENTS ON DOSE INTENSITY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION

There is no question that in most experimental malignancies and in some human tumors, particularly hematological malignancies, high dose intensity treatments have been shown to be superior compared to medium or low intensive therapies. In these malignancies, therefore, utilization of the highest possible dose of the best agents, delivered at the shortest time, will remain valid therapeutic principles governing treatment planning. The very existence of the concept of dose intensity, with the marked limitations of its implementation by bone marrow toxicity, is triggering autologous bone marrow and supportive cytokine research, representing, perhaps, the ultimate extremes in the art and skill of chemotherapy administration.

For most other solid tumors, such as breast, lung, ovary and G.I. malignancies, the beneficial effects of dose intensity on response or survival are far from proven. For these malignancies, it will be essential to stage randomized studies where dose and intensity of the most active agents for a given malignancy is tested as the only variable. The preliminary results of supralethal consolidation treatments utilizing ABMT for solid tumors show, despite impressive complete responses, virtually no impact on the duration of response or length of survival. Therefore, as a result, some investigators already claim that the dose intensity concept may not apply for solid tumors.

To clarify these issues the following points will have to be reexamined in the near future:

1.) Total delivered dose versus dose intensity;
2.) Biological assessments of dose intensity;
3.) Role of maintenance and reinduction therapy.

1.) Total delivered dose versus dose intensity

As discussed by Hryniuk and emphasized by Coppin in this volume, not only the dose intensity, but also the delivery of the total dose is important. The distinction is obvious when one calculates, for instance, the dose intensity of 2 versus 6 treatments, each cycle consisting of equal dose and treatment intervals. In the first instance, while the intensity may be high and equal to the second example, the total delivered dose will be significantly lower than in the second schedule, and hence the total biological therapeutic effect, despite identical dose intensities, will be vastly different.

While the dose intensity will, on account of toxicity, determine the total dose, support therapies such as cytokines or bone marrow transplants may overcome this limitation. In these instances, trials testing in a quantitative manner both entities (the total dose and the intensity) will have to be designed in order to fully clarify the correlation of drug treatment dose with outcome.

2.) Biological assessment of dose intensity

Providing that the uniformity and schedule adherence to the prescribed protocol treatment are both respected good (physician's and patient's compliance the heterogeneity in the magnitude of toxicities of chemotherapy target organs such as bone marrow, G.I. tract or cardiovascular system, will determine the ability of a given patient to receive consistently high intensity chemotherapy treatments within a desired treatment schedule. In patients with increased intrinsic organ sensitivity, the toxicity of the body surface adjusted treatment dose will be significantly higher than in patients with inherent relative resistance to toxic effects of the same chemotherapy dose.

It is proposed that in parallel with organ sensitivity, expressing the in-vivo biological sensitivity to treatment, the tumor cell sensitivity may also be mirrored. In these cases, the treatment delivering the lower dose intensity, providing that the intensity is reduced only as a result of organ toxicity and not deliberately, may be of the same biological anti-tumor effectiveness as the high dose intensity treatment in patients with lesser organ sensitivity. Biological end points such as granulocyte suppression, or degree and duration of alopecia, both measuring the in-vivo effects of delivered chemotherapy, may be essential information milestones to be correlated with both the numerical expression of the quantity of delivered chemotherapeutic intensity, and with the treatment outcome. With such an approach, future trials may be able to fully assess the significance of the measured, delivered dose and the dose intensity.

3.) Role of maintenance and of reinduction therapy

In an attempt to explain the minimal survival impact and short duration of response in solid tumors treated with current programmes using ABMT support, several comments are important. Firstly, it is likely that in the programmes utilizing single consolidation treatment schedule (majority of the ABMT programmes although the instantaneous dose intensity of the consolidation treatment is adequately high, the duration of treatment may be too short to have a meaningful impact on the partially resistant solid tumor stem cells. This may be of importance, particularly in tumors with kinetic rather than biochemical resistance. Taking the long doubling time and relatively low growth fraction of most soft epithelial tumors, it is quite conceivable that times beyond several months will be needed to have all stem cells enter the cell cycle and the dividing state, stages where they may become vulnerable to chemotherapeutic effects. It will be essential to explore the impact of high dose intensity induction, maintenance or pulse re-induction therapies which accompany the massive single consolidation treatment of present ABMT programmes, before the high dose therapy with ABMT support abandoned. Trials such as these, of course, will be less essential for drugs or therapies active against the resting cells, or for malignancies with unusually high growth fraction and a more uniform high sensitivity to a given treatment, such as hematological malignancies.

INTEGRATION OF KINETICS IN TREATMENT PLANNING

As discussed in the first volume, kinetic alterations seen after initial noncurative surgery in experimental systems and documented anecdotally in clinical setting, are of sufficient worry to warrant trials of preoperative therapies, or trials where extent of surgery is minimized. In rationalizing the concept of preoperative chemotherapy, the main arguement put forward indicates that a maximal preoperative reduction of tumor burden at the time of surgery. Shoule be a desired treatment strategy. In minimizing the tumor burden, less viable cells are at risk for adverse kinetic and other effects expected to occur as a result of the noncurative cytoreduction. Also, the exposure of viable macro - or micrometastases to preoperative cytotoxics or differentiating agents may reduce the ability of residual cells to divide or invade more intensely during and mainly after the surgery. Alternatively, preoperatively or postoperatively added therapy utilizing anti- growth factor (GF) treatments, such as monoclonal antibodies, or GF receptor inhibitors, may be of great future relevance. Reduction of the extent and of duration of radical surgeries and general anesthesias, and emphasis on needle rather than of incisional open biopsies, are additional future steps to be explored in this regard.

SHORT-TERM AND LONG-TERM EFFECTS OF THERAPY

As discussed in the last section of this book, the final impact of therapy - the measurable survival gain - will be determined by the overall therapeutic ratio. In this, the cell kill effect of tumor as expressed in tumor response rate and prolongation of the survival, will have to be divided by the side effects of a given treatment. These can be measured and quantified either subjectively in quality life assessments, or objectively as expressed in figures of treatment related morbidity or mortality. The latter toxicity can include such varied entities as days of granulocytopenia and fever, magnitude of G.I. toxicity, alopecia, or induction of second malignancy.

Other organ toxicities are also of importance, such as nephrotoxicity after Cis-platinum or cardiovascular complications such as seen after the use of anthracyclines or with left sided chest wall radiation. In all the decision making processes favouring or denying a given treatment, the recommendation for a treatment should represent documented treatment achieved benefits, anticipated to outweigh its side effects.

The difficulty of carrying this simple statement into daily practice is seen when attempts are made to define precisely the "therapeutic benefit", and to interpret adequately the significance of the toxicities versus treatment gains. These problems are more encountered in less sensitive tumors where therapy does not lead consistently into meaningful responses of cures. To illustrate some of the biases, an example of treatment impact of chemotherapy for extensive oat cell lung carcinoma is shown. In this instance the survival of treated compared to untreated patients is prolonged by 10 months, but no cures are seen. This improvement, if observed in a large patient sample, may be of statistical significance, and does indeed indicate a positive treatment impact. If toxicity is, however, high, and if the survival gain means for an average patient that out of the 10 gained survival months 6 were spent under the duress of chemotherapy, than such an outcome will not code positively on the subjective score of life quality assessment. In this case, the clinicians favouring the routine implementation of such a treatment are biased for the treatment benefits. This, then, is an example of a "statistician bias".

In the illustration of "toxicity" bias, results from adjuvant chemotherapy of node positive postmenopausal breast cancer patients is shown. In this case, a survival gain of approximately 10% is seen as a result of a 6 months course of chemotherapy. To the majority of physicians, and secondarily to their patients, these results indicate that 90 patients must be submitted to cytotoxic treatment for the benefit of the 10, and several peer review reports interpreting these results have indicated, that "the cost of adjuvant chemotherapy for postmenopausal women does not just justify the modest benefits achieved". The actual cost in dollars as well as the subjective cost of toxicity is high. For this reason, a treatment such as this has not been accepted as worthwhile in routine practice. Let us, however, presume, that the 10% survival is truly seen (statistically significant, confirmed in long-term observations, perhaps representing cures). Then it can be calculated that out of the 50,000 newly diagnosed node positive breast cancer patients/year, 5,000 would be saved. Assuming no significant or minimal treatment related mortality and morbidity after adjuvant chemotherapy, it could be stated that the uniform denial of adjuvant chemotherapy for that population of patients may be a result of a "toxicity bias".

These examples illustrate that in addition to a thorough long-term analysis of objective treatment results, skillful interpretation of treatment gains will have to become a part of clinical practice. While at times, such a subjective interpretation is essential, in some cases it may result in group or individual bias.

The value of the therapeutic gain cannot be assessed simply. If short-term or long-term toxicities are substantial and survival gains modest, a thorough scientific, intellectual and moral analysis of achieved results will be essential to popularize the treatments of benefit, and avoid the worrisome, "nevertheless" still presently seen realities of overtreatment with toxic therapies of minimal benefit, while denying, in other instances, treatments of potential value if given to a large proportion of well selected patients.

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EFFECTS OF THERAPY ON BIOLOGY AND KINETICS OF THE RESIDUAL TUMOR, Part B: Clinical Aspects

Proceedings of the International Symposium on the Effects of Therapy on the Biology and Kinetics of the Surviving Tumor Held in Vancouver, British Columbia, Canada, February 15–18, 1989

Joseph Ragaz, Linda Simpson-Herren, Marc E. Lippman, and Bernard Fisher, *Editors*

In the management of cancer, the results of any particular therapeutic approach will vary greatly, not only with a given tumor site, but also among individuals with the same tumor stage and other similar disease characteristics. Effects of Therapy on Biology and Kinetics of the Residual Tumor, Part B: Clinical Aspects examines the effectiveness of different treatment modalities as applied under a range of individual conditions, and discusses the behavior of residual tumors that survive such treatment. Dealing with clinical aspects of therapy on residual tumors, this volume covers the short- and long-term consequences of various techniques on both the host patient as well as the carcinoma itself. This work reviews the following issues in detail: ● Biological response modifiers ● Radiobiology ● Clinical aspects of therapy—integration of dose intensity kinetics and biology with treatment planning ● The role of autologous bone marrow transplantation ● Consequences of therapy.

Effects of Therapy on Biology and Kinetics of the Residual Tumor, Part B: Clinical Aspects presents the most recent work of the world's leading authorities in the field and raises fundamental questions about the biomechanisms of tumor growth in order to point the direction for future research. This comprehensive text will serve as both an up-to-date source of information and a guide to further inquiry for researchers in carcinogenesis, oncology, and biochemistry, as well as clinicians involved in chemotherapy, radiotherapy, or surgery as means of managing cancer growth.

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